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Catalytic Enzyme Activity Concentration in Tissues of Man, Dog, Rabbit, Guinea Pig, Rat and Mouse

Approach to a Quantitative Diagnostic Enzymology, III. Communication

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In memoriam Professor Dr. Dr. Ivar Trautschold († 31. 1. 1984)

Summary: The catalytic activity of up to fifteen enzymes was investigated in the liver, heart, skeletal muscle, kidney (medulla, cortex), brain, lung, duodenum, spleen and pancreas from man and animals. Human specimens were obtained from autopsies and immediately post-mortem from dogs, rabbits, guinea pigs, rats and mice. The differences between our results and previous reports of considerably lower activities for structural enzymes (e. g. creatine kinase) and for enzymes partly of mitochondrial origin (e. g. glutamate dehydrogenase, aspartate aminotransferase, malate dehydrogenase), is attributed to our use of a detergent extraction technique. The superiority of the detergent technique with regard to enzyme yield is exemplified by a comparison of various methods of extraction in rat liver, heart and skeletal muscle. Use of standardized assays allows a qualitative inter-species comparison of results. The influence of autolysis on catalytic activity of human autopsies is considered of minor importance.

Katalytische Enzymaktivitätskonzentration in Geweben von Mensch, Hund, Kaninchen, Meerschweinchen, Ratte und Maus

Versuch der Begründung einer quantitativen Diagnostischen Enzymologie, III.

Zusammenfassung: Das katalytische Aktivitätsmuster von bis zu fünfzehn Enzymen wurde bei Mensch und Tieren für Leber, Herz, Skelettmuskel, Niere (Mark, Rinde), Gehirn, Lunge, Duodenum, Milz und Pankreas erstellt. Die Proben vom Menschen stammten aus Autopsien; Proben von Hund, Kaninchen, Meerschweinchen, Ratte und Maus wurden unmittelbar post mortem gewonnen. Die verglichen mit früheren Ergebnissen beträchtlich höheren Werte, besonders für Enzyme mit mitochondrialen Anteilen (z. B. Glutamatdehydrogenase, Aspartataminotransferase, Malatdehydrogenase) und für strukturinvolvierte Enzyme (z. B. Kreatinkinase), gründen vorrangig auf eine Extraktionstechnik unter Verwendung von Detergenz. Die Überlegenheit der Detergenzanwendung auf die Enzymausbeute wird in einem Vergleich verschiedener Extraktionstechniken beispielhaft für Leber, Herz und Skelettmuskel der Ratte aufgezeigt. Die Verwendung standardisierter Analysemethoden erlaubt einen qualitativen Interspezies-Vergleich. Der Einfluß der Autolyse auf die Ergebnisse der Proben vom Menschen ist von untergeordneter Bedeutung.

Introduction

There are three reasons for investigating the tissue catalytic activity distribution pattern in man and animals.

The first reason is to obtain comprehensive data for enzyme behaviour from the site of origin through the interstitial space and lymph to the bloodstream (1, 2), where they are most commonly sampled for diag-

nostic purposes. In order to compare and determine the relationship of catalytic activity patterns between the intracellular, interstitial, and intravascular spaces identical conditions are required for all analyses. This minimizes the effects of the catalytic changes enzymes go through during their transition from their intracellular origin to their appearance in plasma and subsequent analysis (3).

In clinical enzymology a quantitative approach has been developed over the years to quantify tissue damage in different organs by serial measurements of plasma enzyme catalytic activity. Such quantitative assessment of myocardial damage, for example, has been proposed by several groups (4–6). Similarly, such systems have been devised for localization of liver injury (7). A prerequisite to such an approach is reliable data on tissue enzyme content.

A second reason to quantify tissue enzyme activity is a more general one. The use of commercially available test combinations, which are only occasionally optimized for human plasma conditions, has become increasingly important in human diagnostic enzymology, veterinary medicine, experimental medicine, and also in catalytic activity determination of tissue specimens from man and animals. This development exceeds the original motivation for the development of such tests. The need to compare results from these tests has led to the creation of test standards. With regard to tissue analysis, however, such standardization has by no means led to more homogeneous results. This is mainly due to different tissue preparation and extraction methods, which require further attention and development. A more complete recovery of catalytic activity is obtainable through the use of detergent.

A third reason for this investigation is the paucity of data concerning tissue distribution of enzymes in man and animals, especially in regard to the above-mentioned needs for standardization and more complete extraction techniques.

Surprisingly few comprehensive data on tissue distribution patterns are available for man. Such data is scattered or applies only to a particular enzyme (e. g. creatine kinase (8), lactate dehydrogenase (9)) or to a specific organ. The most elaborate work on liver enzymology to date has been done by the group of E. & F. W. Schmidt, both in man (7, 10, 11) and in animals (12–16).

In addition, to our knowledge, only these authors have established extensive studies on the catalytic activity pattern in human tissues other than the liver (17–19). More or less comprehensive data on tissue

distribution of enzymes in animals are given for the dog (20–24), the rabbit (20, 22, 25), the guinea pig (20, 22, 26), the rat (20, 22, 26–29) and the mouse (22, 26).

Different methods of tissue preparation and/or analysis have led to diverging results which need to be clarified.

For these three reasons, therefore, the catalytic activity distribution pattern of up to fifteen enzymes in nine tissues was established for man, dogs, rabbits, guinea pigs, rats and mice.

Materials and Methods

Man

Twenty autopsies were obtained in patients who died from both non cardiac (12 patients) and cardiac causes (8 patients).

Non cardiac causes were: histiocytoma, aneurysm of base of the brain, dissecting aneurysm, brain infarction, ruptured aorta, brain cancer, hypovolaemic shock, colon cancer, bronchial cancer, tongue cancer. Cardiac causes were: myocardial infarction and acute circulatory and heart failure during surgery.

Patient age ranged from 36 to 79 years, $\bar{x} = 60.2 \pm 11.4$ SD, 17 males, 4 females. Autopsies were done at various times post-mortem (17–42 h) and the following tissue samples were taken: lung (Lobus superior sinister), heart (left ventricle), muscle (M. psoas major), kidney (cortex and medulla), liver (Lobus sinister), spleen, duodenum (cranial part), pancreas, brain (cerebrum, Lobus temporalis).

Tissue samples were taken from areas, which revealed no abnormalities by visual inspection.

Animals

Dogs, rabbits, guinea pigs, rats and mice were used. Strain, housing conditions and diet were described in the first communication of this series (2). Prior to killing, the animals underwent lymph or blood sampling experiments. Animals were sacrificed in accordance with the recommendations of the Universities Federation for Animal Welfare (UFAW) (30).

The following tissue samples were taken immediately:

Dogs

lung (Lobus caudalis sinister), heart (left ventricle), muscle (M. sartorius), kidney (cortex and medulla), liver (Lobus sinister medialis), spleen, duodenum (cranial part), pancreas

Rabbits

lung (Lobus caudalis sinister), heart (left ventricle), muscle (M. sartorius), kidney (cortex and medulla), liver (Lobus sinister medialis), spleen, duodenum (cranial part), pancreas

Rats

lung (Lobus sinister), heart (left ventricle), muscle (M. sartorius), kidney, liver (Lobus sinister medialis), spleen, duodenum (cranial part), pancreas, brain (cerebrum)

Guinea pigs

lung (Lobus sinister), heart (left ventricle), muscle (M. sartorius), kidney, liver (Lobus sinister medialis) spleen, duodenum (cranial part), pancreas, brain (cerebrum)

Mice

lung (Lobus sinister), heart (right and left ventricle), muscle (M. sartorius), kidney, liver (Lobus sinister medialis), spleen, duodenum (cranial part), pancreas, brain (cerebrum)

Tissue samples of about 200–500 mg weight were plunged into ice-cold 0.15 mol/l sodium chloride, freed from connective tissue, carefully dried with soft tissues, and weighed. Until assayed they were stored at -70°C in small, capped, dry containers. Homogenisation was carried out in ice-cold *Krebs-Ringer* buffer containing 10 ml/l of the detergent ethylenepolyethyleneglycol (Nonidet P 40, Fluka AG). This was done three times for 30 s, with intervening intervals of 30 s, by means of an Ultra-Turrax (JANKE + KUNKEL). The ratio of tissue weight to homogenisation buffer volume was 1 : 10. Suitable dilutions were prepared from the 12 000 g supernatant in *Krebs-Ringer* buffer containing detergent.

Determination of catalytic enzyme activity concentration and protein concentration

The catalytic activities of the following enzymes were determined in the tissues:

lactate dehydrogenase (EC 1.1.1.27)
 α -hydroxybutyrate dehydrogenase
 malate dehydrogenase (EC 1.1.1.37)
 isocitrate dehydrogenase (EC 1.1.1.42)
 glutamate dehydrogenase (EC 1.4.1.3)
 aspartate aminotransferase (EC 2.6.1.1)
 alanine aminotransferase (EC 2.6.1.2)
 creatine kinase (EC 2.7.3.2)
 adenylate kinase (EC 2.7.4.3)
 cholinesterase (EC 3.1.1.8)
 alkaline phosphatase (EC 3.1.3.1)
 α -amylase (EC 3.2.1.1)
 amino acid arylamidase (EC 3.4.11.2)
 aldolase (EC 4.1.2.13)
 phosphohexose isomerase (EC 5.3.1.9)

Analytical methods for catalytic enzyme activity concentration and concurrent quality control have been described (2).

α -Hydroxybutyrate dehydrogenase was determined in human tissues according to the Recommendations of the German Society of Clinical Chemistry (31) using a test kit (Boehringer, Mannheim).

Aspartate and alanine aminotransferase were determined in human tissues both with and without supplementary pyridoxal-5'-phosphate (0.1 mmol/l final concentration) and Tris buffer (80 mmol/l, pH 7.8 for aspartate aminotransferase; 100 mmol/l, pH 7.3 for alanine aminotransferase) instead of phosphate buffer, after 1 h incubation. Pyridoxal-5'-phosphate works as a coenzyme and becomes limited in autolysed tissues. Phosphate inhibits the recombination of the apoenzyme with pyridoxal-5'-phosphate (32).

Creatine kinase MB isoenzyme was determined in human heart muscle with the immuno-inhibition method (33) using a test kit (Boehringer, Mannheim).

Soluble protein was determined with the *Folin-Ciocalteu* reagent (35).

In the literature, catalytic activity of tissues is expressed in various ways: catalytic activity per gram wet weight, per dry weight, per DNA, per protein, per nitrogen etc. Several of these methods have been compared by *Hermens et al.* (34). They recommended relation to wet weight of tissues to minimize experimental error. *Schmidt et al.* (10, 12, 17) propose relation to protein concentration as well as wet weight. In the present study catalytic activity was expressed as U/g wet weight and protein concentration as mg/g wet weight. This allows for a rough estimate of specific activity (U/g protein).

Results

The tables 1 a to 1 i summarize the catalytic activities in each tissue for the species investigated. Due to the large number of tables and individual results, we will not attempt to give here a detailed description of all of our finding.

Despite occasional distinct inter-species differences, some general comments as to the tissue distribution of enzymes can be made. Lactate dehydrogenase, malate dehydrogenase, and phosphohexose isomerase are ubiquitously distributed. The highest catalytic activities for α -hydroxybutyrate dehydrogenase are found in heart and striated muscle, followed by the kidney. Significant amounts of isocitrate dehydrogenase are found in the liver and in heart muscle. Glutamate dehydrogenase and alanine aminotransferase were preferentially detected in liver. The latter enzyme was found in the kidney as well. Aspartate aminotransferase exhibited its highest catalytic activities in the heart. Creatine kinase showed the well known spectrum of catalytic activities from skeletal muscle at one end through cardiac muscle and brain to intestine at the other end. Adenylate kinase activities were highest in skeletal muscle. Significant amounts of cholinesterase, alkaline phosphatase, and amino acid arylamidase were found in liver and kidney. Aldolase was mainly found in skeletal muscle.

The catalytic activity fraction of total lactate dehydrogenase catalytic activity in man that was due to α -hydroxybutyrate dehydrogenase was calculated for different areas. These values ($\bar{x} \pm$ standard error mean) were as follows:

liver: $27.4\% \pm 0.32\%$;
 heart muscle: $78.5\% \pm 1.8\%$;
 skeletal muscle: $24.6 \pm 1.1\%$;
 kidney-cortex: $73.0\% \pm 2.4\%$;
 kidney-medulla: $68.0\% \pm 1.9\%$;
 brain: $63.8\% \pm 0.61\%$;
 lung: $51.8\% \pm 2.1\%$;
 duodenum: $47.6\% \pm 1.5\%$;
 spleen: $50.8\% \pm 2.4\%$.

Tab. 1a. Enzyme catalytic activity concentrations (U/g wet weight) and protein concentration (mg/g wet weight) in the liver of man and animals.

Mean $\pm$ standard error mean; n = 20

— not determined, < below the detection limit.

Aspartate aminotransferase and alanine aminotransferase were determined in man without and with the addition of pyridoxal-5'-phosphate (P-5'-P).

	Man	Dog	Rabbit	Guinea pig	Rat	Mouse
Lactate dehydrogenase	152 $\pm$ 7.9	176 $\pm$ 8.1	195 $\pm$ 8.1	80.6 $\pm$ 3.7	517 $\pm$ 13	246 $\pm$ 5.3
α -Hydroxybutyrate dehydrogenase	41.5 $\pm$ 2.1	—	—	—	—	—
Malate dehydrogenase	241 $\pm$ 9.3	305 $\pm$ 18	256 $\pm$ 8.5	492 $\pm$ 19	715 $\pm$ 17	667 $\pm$ 12
Isocitrate dehydrogenase	33.3 $\pm$ 1.3	13.8 $\pm$ 0.72	24.4 $\pm$ 0.76	74.0 $\pm$ 3.3	25.6 $\pm$ 0.20	28.7 $\pm$ 0.32
Glutamate dehydrogenase	157 $\pm$ 8.8	162 $\pm$ 7.6	210 $\pm$ 11.0	333 $\pm$ 11	328 $\pm$ 6.4	282 $\pm$ 5.6
Aspartate aminotransferase	54.3 $\pm$ 3.5	91.0 $\pm$ 4.8	84.5 $\pm$ 2.4	76.3 $\pm$ 2.0	207 $\pm$ 2.4	196 $\pm$ 4.5
Aspartate aminotransferase + P-5'-P	107 $\pm$ 5.4	—	—	—	—	—
Alanine aminotransferase	40.1 $\pm$ 2.8	94.4 $\pm$ 5.4	22.6 $\pm$ 1.5	24.4 $\pm$ 0.56	65.7 $\pm$ 1.3	94.3 $\pm$ 1.7
Alanine aminotransferase + P-5'-P	55.3 $\pm$ 3.1	—	—	—	—	—
Creatine kinase	4.6 $\pm$ 0.62	12.9 $\pm$ 0.63	6.3 $\pm$ 0.41	10.3 $\pm$ 0.48	11.7 $\pm$ 0.27	6.6 $\pm$ 0.13
Adenylate kinase	59.6 $\pm$ 2.2	62.7 $\pm$ 3.2	138 $\pm$ 6.6	70.8 $\pm$ 2.1	192 $\pm$ 2.4	151 $\pm$ 3.8
Choline esterase	0.86 $\pm$ 0.12	14.2 $\pm$ 1.2	5.4 $\pm$ 0.49	27.3 $\pm$ 1.3	1.4 $\pm$ 0.05	6.3 $\pm$ 0.15
Alkaline phosphatase	16.9 $\pm$ 1.5	9.4 $\pm$ 1.6	4.8 $\pm$ 0.23	1.1 $\pm$ 0.05	1.1 $\pm$ 0.03	3.8 $\pm$ 0.15
Amino acid arylamidase	1.7 $\pm$ 0.11	1.1 $\pm$ 0.10	0.54 $\pm$ 0.03	1.5 $\pm$ 0.07	0.89 $\pm$ 0.01	1.3 $\pm$ 0.04
Aldolase	2.7 $\pm$ 0.12	2.8 $\pm$ 0.22	5.1 $\pm$ 0.29	4.0 $\pm$ 0.24	10.2 $\pm$ 0.12	3.8 $\pm$ 0.17
Phosphohexose isomerase	134 $\pm$ 6.3	101 $\pm$ 2.2	72.1 $\pm$ 2.8	93.7 $\pm$ 5.7	99.2 $\pm$ 1.5	93.7 $\pm$ 1.7
Protein	165 $\pm$ 4.4	176 $\pm$ 8.4	244 $\pm$ 7.2	174 $\pm$ 5.0	196 $\pm$ 0.99	174 $\pm$ 3.4

Tab. 1b. Enzyme catalytic activity concentrations (U/g wet weight) and protein concentration (mg/g wet weight) in heart muscle of man and animals.

For further explanation see Tab. 1a.

	Man	Dog	Rabbit	Guinea pig	Rat	Mouse
Lactate dehydrogenase	140 $\pm$ 8.2	280 $\pm$ 16	377 $\pm$ 12	337 $\pm$ 14	528 $\pm$ 8.8	318 $\pm$ 7.0
α -Hydroxybutyrate dehydrogenase	109 $\pm$ 6.5	—	—	—	—	—
Malate dehydrogenase	951 $\pm$ 60	1372 $\pm$ 89	1235 $\pm$ 29	1453 $\pm$ 38	1846 $\pm$ 33	1766 $\pm$ 43
Isocitrate dehydrogenase	11.1 $\pm$ 1.2	21.5 $\pm$ 1.3	42.3 $\pm$ 1.4	59.9 $\pm$ 1.4	69.0 $\pm$ 0.91	58.3 $\pm$ 0.92
Glutamate dehydrogenase	9.8 $\pm$ 0.40	17.9 $\pm$ 0.81	16.9 $\pm$ 0.66	5.2 $\pm$ 0.16	16.7 $\pm$ 0.24	12.8 $\pm$ 0.22
Aspartate aminotransferase	127 $\pm$ 7.0	235 $\pm$ 13	159 $\pm$ 3.7	232 $\pm$ 5.9	352 $\pm$ 5.6	279 $\pm$ 8.3
Aspartate aminotransferase + P-5'-P	200 $\pm$ 11	—	—	—	—	—
Alanine aminotransferase	8.0 $\pm$ 0.7	24.4 $\pm$ 1.1	24.4 $\pm$ 1.4	15.2 $\pm$ 0.62	5.7 $\pm$ 0.18	7.5 $\pm$ 0.27
Alanine aminotransferase + P-5'-P	6.7 $\pm$ 0.5	—	—	—	—	—
Creatine kinase	567 $\pm$ 47	1564 $\pm$ 92	1183 $\pm$ 87	563 $\pm$ 13	856 $\pm$ 15	743 $\pm$ 26
Creatine kinase isoenzyme MB	191 $\pm$ 17	—	—	—	—	—
Adenylate kinase	127 $\pm$ 11	131 $\pm$ 9.2	286 $\pm$ 9.5	84.2 $\pm$ 2.8	279 $\pm$ 7.0	109 $\pm$ 4.2
Choline esterase	0.76 $\pm$ 0.07	1.3 $\pm$ 0.08	1.1 $\pm$ 0.09	0.95 $\pm$ 0.05	3.3 $\pm$ 0.04	1.6 $\pm$ 0.05
Alkaline phosphatase	2.6 $\pm$ 0.30	0.76 $\pm$ 0.08	1.2 $\pm$ 0.06	4.5 $\pm$ 0.14	6.2 $\pm$ 0.14	10.3 $\pm$ 0.57
Amino acid arylamidase	0.12 $\pm$ 0.01	0.15 $\pm$ 0.01	0.32 $\pm$ 0.02	0.47 $\pm$ 0.02	0.36 $\pm$ 0.01	0.65 $\pm$ 0.02
Aldolase	3.6 $\pm$ 0.35	24.5 $\pm$ 0.93	18.6 $\pm$ 0.50	15.7 $\pm$ 0.33	15.9 $\pm$ 0.15	24.3 $\pm$ 0.44
Phosphohexose isomerase	221 $\pm$ 16	184 $\pm$ 6.5	183 $\pm$ 8.1	168 $\pm$ 3.0	141 $\pm$ 3.0	246 $\pm$ 5.7
Protein	92.0 $\pm$ 2.0	102 $\pm$ 11	108 $\pm$ 1.1	116 $\pm$ 1.8	192 $\pm$ 2.5	152 $\pm$ 5.0

Tab. 1c. Enzyme catalytic activity concentrations (U/g wet weight) and protein concentration (mg/g wet weight) in skeletal muscle of man and animals.
For further explanation see Tab. 1a.

	Man	Dog	Rabbit	Guinea pig	Rat	Mouse
Lactate dehydrogenase	230 ± 18	516 ± 44	1270 ± 76	492 ± 22	812 ± 30	577 ± 14
α-Hydroxybutyrate dehydrogenase	55.1 ± 2.8	—	—	—	—	—
Malate dehydrogenase	265 ± 15	593 ± 30	174 ± 12	470 ± 18	476 ± 14	654 ± 8.4
Isocitrate dehydrogenase	6.9 ± 0.44	4.2 ± 0.39	4.0 ± 0.46	8.4 ± 0.44	5.7 ± 0.24	5.0 ± 0.20
Glutamate dehydrogenase	2.0 ± 0.28	1.1 ± 0.16	1.4 ± 0.07	2.8 ± 0.09	3.7 ± 0.04	3.1 ± 0.10
Aspartate aminotransferase	29.6 ± 2.2	142 ± 11	17.9 ± 1.5	95.8 ± 2.2	75.6 ± 1.4	91.4 ± 2.0
Aspartate aminotransferase + P-5'-P	67.4 ± 3.1	—	—	—	—	—
Alanine aminotransferase	6.2 ± 0.39	7.8 ± 0.53	2.5 ± 0.30	26.0 ± 0.85	9.0 ± 0.17	5.8 ± 0.17
Alanine aminotransferase + P-5'-P	6.8 ± 0.33	—	—	—	—	—
Creatine kinase	2949 ± 180	2884 ± 129	5768 ± 400	3906 ± 114	5319 ± 76	3694 ± 92
Adenylate kinase	166 ± 16	368 ± 37	859 ± 54	205 ± 8.5	886 ± 13	850 ± 34
Choline esterase	0.31 ± 0.03	0.42 ± 0.02	0.35 ± 0.02	0.69 ± 0.02	0.29 ± 0.01	0.59 ± 0.03
Alkaline phosphatase	0.40 ± 0.03	0.67 ± 0.06	0.18 ± 0.01	1.5 ± 0.22	0.69 ± 0.01	3.4 ± 0.11
Amino acid arylamidase	0.18 ± 0.01	0.08 ± 0.01	0.25 ± 0.01	0.47 ± 0.01	0.26 ± 0.01	0.20 ± 0.01
Aldolase	85.3 ± 4.1	72.3 ± 6.0	301 ± 19	102 ± 3.8	134 ± 7.9	149 ± 3.7
Phosphohexose isomerase	138 ± 7.9	241 ± 17	889 ± 49	355 ± 8.0	411 ± 6.9	347 ± 5.9
Protein	86.5 ± 2.0	80.8 ± 3.4	95.3 ± 3.1	76.2 ± 2.3	83.1 ± 1.1	96.8 ± 1.6

Tab. 1d. Enzyme catalytic activity concentrations (U/g wet weight) and protein concentration (mg/g wet weight) in the lung of man and animals.
For further explanation see Tab. 1a.

	Man	Dog	Rabbit	Guinea pig	Rat	Mouse
Lactate dehydrogenase	38.3 ± 3.0	50.0 ± 1.9	39.3 ± 1.2	51.7 ± 1.8	77.5 ± 2.1	75.4 ± 2.1
α-Hydroxybutyrate dehydrogenase	19.1 ± 1.1	—	—	—	—	—
Malate dehydrogenase	45.6 ± 2.5	67.0 ± 3.9	75.9 ± 2.3	204 ± 13	167 ± 6.5	219 ± 5.1
Isocitrate dehydrogenase	1.2 ± 0.12	1.8 ± 0.12	5.1 ± 0.18	5.2 ± 0.12	5.0 ± 0.16	3.4 ± 0.07
Glutamate dehydrogenase	3.1 ± 0.30	12.0 ± 0.35	8.3 ± 0.47	13.8 ± 0.32	<	10.0 ± 0.15
Aspartate aminotransferase	2.4 ± 0.41	9.8 ± 0.41	8.1 ± 0.33	20.1 ± 0.42	15.3 ± 0.56	17.0 ± 0.25
Aspartate aminotransferase + P-5'-P	4.7 ± 0.50	—	—	—	—	—
Alanine aminotransferase	0.46 ± 0.06	0.32 ± 0.03	3.6 ± 0.12	1.2 ± 0.07	3.4 ± 0.20	3.0 ± 0.05
Alanine aminotransferase + P-5'-P	0.44 ± 0.08	—	—	—	—	—
Creatine kinase	10.9 ± 1.1	20.6 ± 2.4	19.5 ± 1.2	35.4 ± 0.82	43.2 ± 1.8	41.0 ± 0.93
Adenylate kinase	22.8 ± 2.2	24.9 ± 0.68	40.3 ± 1.3	26.4 ± 1.1	34.0 ± 1.0	32.9 ± 1.2
Choline esterase	0.88 ± 0.12	1.3 ± 0.10	0.94 ± 0.08	2.1 ± 0.10	1.2 ± 0.04	1.4 ± 0.03
Alkaline phosphatase	4.0 ± 0.74	3.5 ± 0.37	19.5 ± 1.1	14.0 ± 0.81	24.7 ± 0.99	8.4 ± 0.44
Amino acid arylamidase	0.40 ± 0.04	1.3 ± 0.06	0.37 ± 0.02	1.8 ± 0.03	0.69 ± 0.05	0.4 ± 0.02
Aldolase	1.1 ± 0.08	2.1 ± 0.10	2.7 ± 0.08	4.8 ± 0.13	3.5 ± 0.12	3.5 ± 0.08
Phosphohexose isomerase	35.5 ± 2.2	51.2 ± 1.9	43.6 ± 1.3	81.4 ± 1.5	64.7 ± 1.6	80.5 ± 1.4
Protein	107 ± 3.5	117 ± 7.2	117 ± 1.8	159 ± 3.8	152 ± 8.0	135 ± 2.0

Tab. 1e. Enzyme catalytic activity concentrations (U/g wet weight) and protein concentration (mg/g wet weight) in the spleen of man and animals.
For further explanation see Tab. 1a.

	Man	Dog	Rabbit	Guinea pig	Rat	Mouse
Lactate dehydrogenase	91.0 ± 8.0	79.0 ± 4.5	70.7 ± 2.3	98.4 ± 1.9	116 ± 1.8	105 ± 1.9
α-Hydroxybutyrate dehydrogenase	45.3 ± 3.6	—	—	—	—	—
Malate dehydrogenase	111 ± 9.0	99.4 ± 7.1	137 ± 4.2	148 ± 3.2	170 ± 4.9	256 ± 5.5
Isocitrate dehydrogenase	2.0 ± 0.16	2.5 ± 0.21	7.4 ± 0.20	4.2 ± 0.40	3.3 ± 0.09	5.4 ± 0.12
Glutamate dehydrogenase	11.1 ± 0.93	8.8 ± 0.56	19.6 ± 0.96	8.2 ± 0.24	12.0 ± 0.37	15.3 ± 0.50
Aspartate aminotransferase	2.7 ± 0.25	12.6 ± 0.60	14.4 ± 0.41	20.0 ± 0.48	22.8 ± 0.86	19.3 ± 0.35
Aspartate aminotransferase + P-5'-P	8.3 ± 0.30	—	—	—	—	—
Alanine aminotransferase	0.55 ± 0.06	0.30 ± 0.02	0.31 ± 0.02	1.9 ± 0.04	0.97 ± 0.02	0.76 ± 0.04
Alanine aminotransferase + P-5'-P	0.40 ± 0.06	—	—	—	—	—
Creatine kinase	3.4 ± 0.36	46.9 ± 2.5	19.6 ± 0.73	68.1 ± 2.3	44.7 ± 0.90	32.2 ± 0.88
Adenylate kinase	38.1 ± 2.2	31.4 ± 1.1	10.5 ± 0.25	24.6 ± 0.52	33.8 ± 0.45	29.9 ± 0.64
Choline esterase	0.54 ± 0.05	0.72 ± 0.05	1.5 ± 0.16	1.3 ± 0.09	2.7 ± 0.09	0.93 ± 0.05
Alkaline phosphatase	9.0 ± 1.1	1.0 ± 0.13	23.3 ± 1.2	9.2 ± 0.65	0.59 ± 0.02	6.81 ± 0.14
Amino acid arylamidase	0.73 ± 0.06	2.3 ± 0.17	1.5 ± 0.09	1.6 ± 0.06	0.90 ± 0.02	1.32 ± 0.04
Aldolase	2.3 ± 0.16	1.8 ± 0.11	2.1 ± 0.15	4.6 ± 0.14	3.6 ± 0.09	2.9 ± 0.13
Phosphohexose isomerase	90.3 ± 7.6	66.1 ± 4.7	90.9 ± 2.9	113 ± 3.9	79.0 ± 2.6	99.1 ± 1.5
Protein	173 ± 5.0	187 ± 12	119 ± 3.8	126 ± 4.4	258 ± 5.7	147 ± 2.5

Tab. 1f. Enzyme catalytic activity concentrations (U/g wet weight) and protein concentration (mg/g wet weight) in the duodenum of man and animals.
For further explanation see Tab. 1a.

	Man	Dog	Rabbit	Guinea pig	Rat	Mouse
Lactate dehydrogenase	66.5 ± 4.9	76.6 ± 4.8	45.4 ± 3.5	94.4 ± 2.3	297 ± 6.8	297 ± 11
α-Hydroxybutyrate dehydrogenase	31.8 ± 2.7	—	—	—	—	—
Malate dehydrogenase	99.0 ± 7.9	167 ± 13	146 ± 7.6	493 ± 17	683 ± 19	291 ± 19
Isocitrate dehydrogenase	3.0 ± 0.26	4.2 ± 0.25	5.4 ± 0.33	21.4 ± 1.0	12.9 ± 0.27	6.2 ± 0.44
Glutamate dehydrogenase	5.2 ± 0.30	18.2 ± 1.6	19.2 ± 1.3	17.1 ± 0.44	25.6 ± 1.0	5.6 ± 0.86
Aspartate aminotransferase	6.7 ± 0.53	19.5 ± 0.85	12.9 ± 0.72	35.5 ± 0.76	33.7 ± 0.70	32.9 ± 0.70
Aspartate aminotransferase + P-5'-P	16.3 ± 0.99	—	—	—	—	—
Alanine aminotransferase	1.2 ± 0.12	0.70 ± 0.11	6.1 ± 0.32	6.9 ± 0.29	48.0 ± 1.7	19.5 ± 1.6
Alanine aminotransferase + P-5'-P	1.6 ± 0.16	—	—	—	—	—
Creatine kinase	66.9 ± 4.4	258 ± 16	140 ± 11	257 ± 9.5	229 ± 6.6	110 ± 5.5
Adenylate kinase	21.3 ± 1.9	55.5 ± 2.5	66.0 ± 2.3	62.7 ± 2.5	115 ± 3.5	87.5 ± 14
Choline esterase	1.6 ± 0.12	3.5 ± 0.41	2.1 ± 0.13	15.2 ± 1.0	11.4 ± 0.23	14.2 ± 0.61
Alkaline phosphatase	15.6 ± 1.7	31.5 ± 5.0	15.6 ± 2.2	30.8 ± 1.6	500 ± 17	552 ± 15
Amino acid arylamidase	1.2 ± 0.15	1.4 ± 0.16	0.29 ± 0.03	3.4 ± 0.11	1.8 ± 0.04	2.6 ± 0.14
Aldolase	1.9 ± 0.12	4.7 ± 0.31	2.9 ± 0.20	8.8 ± 0.27	0.91 ± 0.08	0.36 ± 0.04
Phosphohexose isomerase	65.8 ± 6.7	66.6 ± 3.8	57.5 ± 3.4	124 ± 2.3	138 ± 4.1	206 ± 5.2
Protein	95.4 ± 3.6	92.1 ± 5.7	117 ± 6.6	127 ± 2.6	126 ± 6.4	74.7 ± 3.1

Tab. 1g. Enzyme catalytic activity concentrations (U/g wet weight) and protein concentration (mg/g wet weight) in the kidney of man and animals.
For further explanation see Tab. 1a.

	Man		Dog		Rabbit		Guinea pig	Rat	Mouse
	cortex	medulla	cortex	medulla	cortex	medulla			
Lactate dehydrogenase	164 ± 4.8	123 ± 10	297 ± 17	73.0 ± 5.3	89.6 ± 3.1	99.7 ± 7.6	96.9 ± 4.4	181 ± 10	324 ± 6.1
α-Hydroxybutyrate dehydrogenase	120 ± 4.6	84.7 ± 7.8	—	—	—	—	—	—	—
Malate dehydrogenase	219 ± 9.5	168 ± 14	490 ± 29	83.0 ± 8.9	474 ± 9.4	217 ± 23	699 ± 9.6	854 ± 7.2	662 ± 1.1
Isocitrate dehydrogenase	4.6 ± 0.46	2.2 ± 0.24	11.9 ± 0.49	1.8 ± 0.23	42.8 ± 1.3	13.6 ± 1.6	49.3 ± 1.3	43.3 ± 0.60	42.0 ± 0.96
Glutamate dehydrogenase	43.5 ± 3.4	31.7 ± 4.5	61.8 ± 3.4	6.6 ± 0.80	40.1 ± 1.4	8.6 ± 0.91	46.7 ± 1.4	199 ± 2.5	76.6 ± 1.4
Aspartate aminotransferase	14.6 ± 1.3	12.0 ± 1.2	55.2 ± 2.7	13.2 ± 1.5	42.7 ± 0.91	17.5 ± 2.0	78.8 ± 1.7	115 ± 2.0	107 ± 2.0
Aspartate aminotransferase + P-5'-P	34.1 ± 1.7	28.6 ± 2.8	—	—	—	—	—	—	—
Alanine aminotransferase	7.9 ± 0.78	3.8 ± 0.48	10.0 ± 0.74	0.26 ± 0.04	16.3 ± 0.81	22.8 ± 4.0	10.8 ± 0.44	1.8 ± 0.06	6.1 ± 0.11
Alanine aminotransferase + P-5'-P	9.1 ± 1.4	4.4 ± 0.50	—	—	—	—	—	—	—
Creatine kinase	8.0 ± 1.2	6.4 ± 1.1	9.5 ± 0.48	1.3 ± 0.22	16.7 ± 0.93	12.7 ± 1.2	21.3 ± 0.61	28.7 ± 1.1	11.7 ± 0.57
Adenylate kinase	54.8 ± 4.8	30.8 ± 3.2	90.0 ± 5.2	21.7 ± 1.6	176 ± 6.7	45.5 ± 4.7	98.6 ± 2.1	180 ± 2.4	128 ± 2.7
Choline esterase	0.37 ± 0.02	0.44 ± 0.04	2.2 ± 0.10	1.8 ± 0.16	1.0 ± 0.05	0.31 ± 0.02	2.1 ± 0.04	0.69 ± 0.02	1.2 ± 0.02
Alkaline phosphatase	15.9 ± 1.4	11.5 ± 1.6	0.75 ± 0.07	0.74 ± 0.14	41.8 ± 2.7	7.0 ± 1.0	156 ± 1.1	153 ± 2.1	296 ± 10
Amino acid arylamidase	10.3 ± 0.53	6.6 ± 0.91	3.7 ± 0.24	0.13 ± 0.01	10.5 ± 0.69	1.2 ± 0.22	19.1 ± 1.1	10.6 ± 0.15	3.7 ± 0.14
Aldolase	2.4 ± 0.22	1.7 ± 0.16	3.2 ± 0.13	1.8 ± 0.15	7.1 ± 0.21	3.2 ± 0.21	40.8 ± 1.4	7.9 ± 0.28	10.8 ± 0.47
Phosphohexose isomerase	74.3 ± 6.4	52.3 ± 4.7	113 ± 5.8	57.2 ± 4.2	115 ± 3.5	96.0 ± 8.1	109 ± 6.0	161 ± 2.9	203 ± 6.2
Protein	129 ± 2.9	120 ± 3.7	148 ± 9.9	75.0 ± 3.8	200 ± 3.2	153 ± 5.8	128 ± 3.3	172 ± 3.0	182 ± 4.0

Tab. 1h. Enzyme catalytic activity concentrations (U/g wet weight) and protein concentration (mg/g wet weight) in the brain of man and animals.
For further explanation see Tab. 1a.

	Man		Guinea pig		Rat		Mouse	
Lactate dehydrogenase	102 ± 1.5	—	115 ± 5.1	137 ± 1.6	146 ± 2.3	—	—	—
α-Hydroxybutyrate dehydrogenase	64.8 ± 1.0	—	—	—	—	—	—	—
Malate dehydrogenase	347 ± 8.0	—	498 ± 12	537 ± 13	806 ± 30	—	—	—
Isocitrate dehydrogenase	1.6 ± 0.16	—	3.7 ± 0.09	1.8 ± 0.08	3.0 ± 0.11	—	—	—
Glutamate dehydrogenase	30.5 ± 0.60	—	27.2 ± 1.0	31.3 ± 0.41	31.7 ± 0.42	—	—	—
Aspartate aminotransferase	22.4 ± 1.5	—	120 ± 3.5	113 ± 1.9	138 ± 2.7	—	—	—
Aspartate aminotransferase + P-5'-P	54.8 ± 1.4	—	—	—	—	—	—	—
Alanine aminotransferase	1.0 ± 0.09	—	3.7 ± 0.14	3.4 ± 0.09	3.6 ± 0.14	—	—	—
Alanine aminotransferase + P-5'-P	1.4 ± 0.18	—	—	—	—	—	—	—
Creatine kinase	193 ± 9.5	—	557 ± 18	365 ± 7.7	500 ± 7.0	—	—	—
Adenylate kinase	24.0 ± 2.1	—	101 ± 6.7	109 ± 2.7	112 ± 2.0	—	—	—
Choline esterase	0.17 ± 0.01	—	2.4 ± 0.14	0.75 ± 0.03	0.94 ± 0.03	—	—	—
Alkaline phosphatase	2.0 ± 0.13	—	3.4 ± 0.39	1.8 ± 0.06	2.4 ± 0.08	—	—	—
Amino acid arylamidase	0.30 ± 0.05	—	0.31 ± 0.02	0.29 ± 0.01	0.30 ± 0.01	—	—	—
Aldolase	11.5 ± 0.75	—	22.6 ± 0.46	21.5 ± 0.28	21.2 ± 0.25	—	—	—
Phosphohexose isomerase	129 ± 7.7	—	164 ± 3.7	197 ± 2.5	245 ± 3.4	—	—	—
Protein	78.9 ± 1.9	—	131 ± 3.5	175 ± 5.7	125 ± 1.6	—	—	—

Tab. 1i. α-Amylase catalytic activity concentration (U/g wet weight) and protein concentration (mg/g wet weight) in the pancreas of man and animals.
For further explanation see Tab. 1a.

	Man		Dog		Rabbit		Guinea pig		Rat		Mouse	
α-Amylase	2605 ± 260	2431 ± 163	2431 ± 221	2311 ± 127	3449 ± 349	3497 ± 1910	10578 ± 570	39497 ± 1910	—	—	—	—
Protein	93.7 ± 3.4	154 ± 5.4	154 ± 1.7	194 ± 6.6	136 ± 8.9	170 ± 5.6	130 ± 8.9	170 ± 5.6	—	—	—	—

The degree to which addition of pyridoxal-5'-phosphate increased aspartate aminotransferase activity in human autopsy specimens is given below for different organs. Results are reported as fractional increases ($\bar{x} \pm$ standard error mean); significance values for paired t-tests are also reported:

liver: $107\% \pm 11.8\%$, $p < 0.01$;
heart muscle: $62.8\% \pm 9.3\%$, $p < 0.05$;
skeletal muscle: $143\% \pm 12.6\%$, $p < 0.01$;
kidney-cortex: $170\% \pm 29.3\%$, $p < 0.01$;
kidney-medulla: $154\% \pm 22.6\%$, $p < 0.01$;
brain: $158\% \pm 13.0\%$, $p < 0.01$;
lung: $95.2\% \pm 9.1\%$, $p < 0.05$;
duodenum: $155\% \pm 15.9\%$, $p < 0.01$;
spleen: $314\% \pm 60.1\%$, $p < 0.001$.

For alanine aminotransferase the fractional increases or decreases from pyridoxal-5'-phosphate were as follows:

liver: $+35.2\% \pm 5.1\%$, n. s.;
heart muscle: $-12.8\% \pm 4.9\%$, n. s.;
skeletal muscle: $+14.4\% \pm 5.7\%$, n. s.;
kidney-cortex: $+21.4\% \pm 10.8\%$, n. s.;
kidney-medulla: $+8.4\% \pm 3.4\%$, n. s.;
brain: $+63.2\% \pm 25.1\%$, n. s.;
lung: $+12.7\% \pm 12.2\%$, n. s.;
duodenum: $+39.4\% \pm 14.7\%$, n. s.;
spleen: $-19.5\% \pm 12.8\%$, n. s.

The catalytic activity fraction of creatine kinase MB isoenzyme to total creatine kinase catalytic activity in human heart muscle, calculated from individual values was $36.7\% \pm 2.9\%$ ($\bar{x} \pm$ standard error mean):

As enzymes from the tissues preferentially reach the intravascular space via lymphatic transport (1), one can argue that the catalytic activity pattern in the isolated organ lymph from liver and intestine correlate with the respective tissue activity pattern in these organs. However, a comparison of the activity pattern of intestinal lymph with the activity pattern itself from the dog, the rabbit and the rat yield correlation coefficients of 0.179, 0.893 and 0.280, respectively. A similar comparison of liver lymph and liver tissue patterns in the dog and the rabbit gave correlation coefficients of 0.161 and 0.403, respectively. Lymph catalytic activity data were taken from part II of this series (1).

Discussion

The gradual improvement and standardization of assay conditions in the last 10–20 years has resulted in a steadily increasing homogeneity and comparability of results, at least for human plasma.

Absolute values for tissue enzyme content as reported by different authors, however, paint a confusing picture and differ from one another sometimes by as much as a factor of 10.

There are three main aspects which contribute to these large differences:

- (1) Effect of various methods of tissue disintegration on extraction of enzymes and protein. This is especially true, as will be shown, when detergent is used.
- (2) Effect of the enzyme test conditions employed. Finally, as human tissue samples are often obtained at autopsy (as in our present study) we have to consider:
- (3) The degree of comparability of autopsy vs. biopsy samples.

These are discussed in the following.

Ad 1 There exist a great variety of extraction techniques. The most frequently used are: *Potter-Elvehjem* homogenizer (glass-teflon), blade homogenizers (e. g. *Ultra-Turrax*), and fractionated extraction (*Bücher*). All have been thoroughly compared with respect to human liver disintegration (36). Stabilizing reagents and activators like mercaptoethanol, dithioerythritol and pyridoxal-5'-phosphate were introduced later. Various detergents have been used (37–43), but the claim that these improve the extraction of mitochondrial enzymes is poorly documented. In blood cells systematic studies on the varying cell-disruption techniques and application of detergent have been carried out (for review see: l. c. (44)). To our knowledge such studies for parenchymatous tissues are largely lacking. *Mühlhaus* (45) has shown the advantageous properties of detergent on recovery of nine enzymes from rat heart, but studies of other tissues have not yet been done. This relative paucity of data led us to investigate more elaborately the different combinations of extraction techniques and their effect on the yield of currently investigated enzymes and on protein in rat skeletal muscle, heart and liver. The results are shown for some enzymes in figure 1, where catalytic activity and mass concentration fractions are compared with a reference or "gold-standard" procedure. In every case tissues were disintegrated by means of an *Ultra-Turrax* (3 times for 30 s, see Materials and Methods) in *Krebs-Ringer* buffer with and without addition of 10 ml/l detergent Nonidet (NP-40). Sonification was performed at 30 W (2 times for 2 min in the same tube, cooled in ice-water). Appropriate dilutions for assays were prepared with *Krebs-Ringer* buffer with and without the addition of detergent (10 ml/l). The results can be summarized as follows:

Fig. 1. Catalytic activity of enzymes and protein concentration in rat skeletal muscle (a), liver (b) and heart (c) by different combinations of extraction techniques as compared to a reference procedure set as 100%. Mean values of four animals; parallel estimates of the same specimens. In every case tissues were disintegrated by means of an Ultra-Turrax (3 times for 30 s, see Material and Methods) in *Krebs-Ringer* buffer with and without addition of 10 ml/l detergent Nonidet, NP 40. Sonification was performed at 30 W (2 times for 2 min in the same tube, cooled in ice-water). Appropriate dilutions for assays were prepared with *Krebs-Ringer* buffer with and without the addition of detergent (10 ml/l).

a) The use of detergent as a supplement to both homogenisation and dilution media yields the highest activity for every enzyme investigated as well as for protein. This recommended procedure results in the following values (enzyme fractions) for enzymes not shown in the graph. Results are given for muscle, liver, and heart, respectively:

isocitrate dehydrogenase 138%, 112%, 146%;
alanine aminotransferase 114%, 105%, 101%;
adenylate kinase 158%, 166%, 158%;
aldolase 102%, 101%, 101%;
phosphohexose isomerase 103%, 108%, 117%.

Not investigated were cholinesterase, alkaline phosphatase, and amino acid arylamidase.

b) Sonification alone results in only a small fractional increase, whereas addition of detergent to the extraction and/or dilution buffer increases enzyme yield. This points to the desorbing effect of detergent, which prevents reassociation of solubilized enzyme with cell debris.

c) The increase in extractable catalytic activity is positively correlated with the degree to which the particular enzyme is localized in the mitochondria.

The catalytic activity fraction of mitochondrial glutamate dehydrogenase with respect to total catalytic activity in rat liver, heart and skeletal muscle has been reported as 80–100%, 13–65%, and 50%, respectively (13, 20, 41, 46).

The values for aspartate aminotransferase were 59–84% for the liver, 37% for the cardiac muscle, and 10% for skeletal muscle (13, 20, 46–48). For malate dehydrogenase the values were 21–45%, 35%, 63%, respectively (13, 45–47, 49).

Unfortunately extraction techniques differ. Except for the techniques proposed by *McDaniel* et al. (41) and *Mühlhaus* (45), detergent is not routinely used. Reported values probably, therefore, rather underestimate mitochondrial enzyme contributions. It is not surprising then that the greatest differences in total catalytic activity between our results and those in the literature are for enzymes with mitochondrial contributions. These catalytic activity differences are 2 to 3-fold in studies of animal tissues where no autolysis has occurred and in which identical analytical methods to ours were used (20, 21, 26, 50, 51).

Creatine kinase has been found in the mitochondria of the heart, skeletal muscle and brain of vertebrates. The relative proportions vary considerably from species to species and with the extraction procedure employed. These ranges are 3–10%, and 3–30% for skeletal muscle and heart, respectively (20, 38, 52).

In the rat heart, especially, creatine kinase catalytic activity seems to be associated with various fine structures of the cell and no catalytic activity can be detected in the cytoplasm (53). Consistent with this localization is our finding that creatine kinase activity in muscle tissue and brain of various animals is 2 to 4-fold higher than reported previously (20, 21).

For such a comparison the "old", glutathione-activated creatine kinase assay, was transformed by a coefficient of 1.49 and/or a temperature coefficient (25–37 °C) of 2.32 (54).

These differences can hardly be attributed to the different fibre type composition (55–57), nor to different muscles (58), nor to different sites of the heart or transmural gradients (59). There was a difference even for lactate dehydrogenase (20, 21), commonly referred to as a soluble enzyme. This could be due to the fact that isoenzymes lactate dehydrogenase 1 and 2 are attached to the mitochondria (60).

d) Detergent supplementation of extraction and dilution buffer at the described concentration did not alter catalytic enzyme activity in and of itself. Addition of detergent to particle-free tissue supernatants of heart, liver or skeletal muscle (170 000 g, 15 min, Airfuge, Beckman Instruments) disintegrated in buffer only, did not affect catalytic activity in either direction. The same held true for the addition of detergent to plasma and comparative catalytic activity determinations (results not shown).

e) We are aware that disintegration with an Ultra-Turrax may lead to partial inhibition of cytosolic enzymes. This has been documented in comparison with a *Potter-Elvehjem* homogenate (36). This phenomenon, however, may also be explained by partial reassociation of soluble enzyme with subcellular particles. Such reassociation depends on the ionic strength and osmolarity of the medium (61). Such reassociation should be avoided by dilution of supernatant, which is by no means particle-free, with detergent containing buffer for the assay.

Ad 2 Enzymes exist in genetically distinct molecular forms in mammalian tissues. These isoenzymes differ in their catalytic and immunological properties, amino acid composition, and physical characteristics in different tissues of the same species and between species. A fixed assay can therefore never fulfill the impossible demand of always being optimized. This is true even for measurements in human plasma or serum, for which these so-called optimized methods have been developed. The term "optimized" can only be interpreted to apply to a general set of conditions, such as substrate concentrations, buffer composition,

pH, temperature, coenzymes, activators etc. which describes the most satisfactory conditions for the measurement of catalytic activity in a variety of human plasma/serum specimens. These conditions were developed largely with the diagnosis of liver and cardiac disease in mind. It is reasonable to use optimization techniques for lactate dehydrogenase, aspartate aminotransferase, and other enzymes (62, 63), in order to study different tissues of different species, with the caveat that strict quantitative comparisons are not always valid.

In the present study, the currently available "optimized" tests as recommended by the Deutsche Gesellschaft für Klinische Chemie (64) were applied. Additionally, for aspartate and alanine aminotransferase determination we followed as closely as possible provisional IFCC reference methods (65, 66) by using pyridoxal-5'-phosphate and Tris buffer instead of phosphate buffer in the recommended concentration. Pyridoxal-5'-phosphate-induced stimulation of aspartate aminotransferase has been shown to be 26% in myocardial biopsies and 112% in myocardial autopsy specimens (32). Our study reveals an even higher stimulation for some other tissues. It has been argued that the underlying mechanism of this pyridoxal-5'-phosphate effect is a deficiency of pyridoxal-5'-phosphate in autolysing tissues (32). This theory does not explain, however, why alanine aminotransferase, which, like aspartate aminotransferase requires pyridoxal-5'-phosphate as coenzyme, did not show any significant stimulation in our study.

Ad 3 Autolytic alterations affect different catalytic activities differently, depending on the particular tissue, the species, and the analytical method (10, 17, 32, 67). Generally, human heart and skeletal muscle can sustain autolysis for up to 50 h without significant deterioration of the enzyme pattern. This is true for lactate dehydrogenase, α -hydroxybutyrate dehydrogenase, malate dehydrogenase, alanine aminotransferase and phosphohexose isomerase (17, 34, 68). The loss in catalytic activity of aspartate aminotransferase (34) was prevented by the addition of pyridoxal-5'-phosphate to the extraction (69) or incubation buffer (32).

The introduction of the N-acetylcysteine-activated creatine kinase assay also seems to avoid inactivation to a greater extent than seen with the glutathione-activated method (34, 69). The absolute creatine kinase values for autopsy specimens from our study are generally higher as compared to former studies using biopsies (54, 58, 70–73), a finding arguing indirectly against a marked autolytic change for this enzyme in heart and skeletal muscle. Higher values

have been reported only by *Haralambie & Uy* (74) for human left papillary muscle. This is probably due to the well known differences between the various functional areas of the heart (73). The use of detergent in the present study also contributed, to an unknown degree, to the finding of such high catalytic activities.

Liver and pancreas, in contrast, would be expected to undergo more significant autolytic changes due to their high proteolytic capacity. Unfortunately, the catalytic activities of surgically obtained human liver biopsies and autopsy specimens have only rarely been directly compared (10). A thorough review of the few studies concerning this problem reveals a wide variety of results obtained by different authors (10). Nevertheless, we found similar results for both autopsy samples and biopsy samples for lactate dehydrogenase, isocitrate dehydrogenase, aspartate aminotransferase, alanine aminotransferase and creatine kinase. For aldolase our autopsy values were lower, and for malate dehydrogenase and glutamate dehydrogenase our autopsy values were higher (10, 18, 19, 43, 75).

Our attempt to relate the established catalytic activity patterns of liver and intestine of the dog, the rabbit and the rat with patterns in the lymph was not conclusive. Correlation coefficients varied widely. *Friedel et al.* (76) also failed to find in rats any relation between intestinal lymph and the duodenum, jejunum or mesenteric lymph nodes. He gave several possible explanations for this lack of correlation.

He postulated that enzymes could alter their catalytic activity during the release from the intracellular into the interstitial space by means of a "sudden effect" (3, 77). He also theorized that perhaps enzymes could originate from distinct cell types which are masked by the activity pattern of the whole organ and that enzymes could undergo various proteolytic cleavages before entering a lymph vessel.

The poor correlation between catalytic activity pattern of lymph and the respective tissue, however, is markedly improved if only cytosolic enzymes are considered. It is well known that mitochondrial and cytosolic (iso)enzymes are released in different amounts under physiological and certain pathological conditions.

The release pattern of all cytosolic enzymes from the perfused heart is strongly related to their respective intracellular amount (78). The underproportional release of mitochondrial enzymes, however, may be explained by an "accident": mitochondrial enzymes, synthesized in the cytosolic compartment are released like cytosolic enzymes until they have reached the mitochondrial compartment.

After myocardial infarction, liver damage and several in vitro injury conditions, mitochondrial isoenzymes are not released to the same extent as their cytosolic isoenzymes (43, 63, 79–82).

Methods have been reported for the estimation of infarct size (4–6) and for the intralobular localization of liver injury (7, 83) by serial measurement of

plasma catalytic activity. Nevertheless, reliable data on tissue enzyme content are a prerequisite for such a quantitative approach.

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References

- Lindena, J., Küpper, W. & Trautschold, I. † (1986) *this J.* 24, 19–33.
- Lindena, J. & Trautschold, I. † (1986) *this J.* 24, 11–18.
- Schmidt, E., Schmidt, F. W., Herfarth, C., Dettmar, K. H. (1966) *Enzym. Biol. Clin.* 7, 53–72.
- Willems, G. M., Muijtjens, A. M., Lambi, F. H. & Hermens, W. T. (1979) *Cardiovasc. Res.* 13, 578–587.
- Norris, R. M., Howell, D., Whitlock, R. M., Heng, M. K. & Peter, T. (1976) *Eur. J. Cardiol.* 4, 461–468.
- Sobel, B. E., Markham, J. & Roberts, R. (1977) *Am. J. Cardiol.* 39, 130–132.
- Schmidt, E. & Schmidt, F. W. (1979) Enzyme diagnosis in diseases of the liver and the biliary system. In: *Advances in Clinical Enzymology* (Schmidt, E., Schmidt, F. W., Trautschold, I. & Friedel, R., eds.). Karger, Basel pp. 239–292.
- Lang, H. (ed.) (1981) Creatine kinase isoenzymes. Pathophysiology and clinical application. Springer Verlag, Berlin.
- Pfleiderer, G. & Wachsmuth, E. G. (1961) *Biochem. Z.* 334, 185–198.
- Schmidt, E. & Schmidt, F. W. (1970) *Enzym. Biol. Clin.* 11, 67–129.
- Schmidt, E. & Schmidt, F. W. (1976) *FEBS Letters* 62, Supplement E 62-E 79.
- Schmidt, E., Schmidt, F. W. & Wildhirt, E. (1958) *Klin. Wochenschr.* 36, 172–176.
- Schmidt, E. & Schmidt, F. W. (1963) *Enzym. Biol. Clin.* 3, 73–79.
- Schmidt, E., Schmidt, F. W., Herfarth, C., Dettmar, K. H. & Fabel H. (1966) *Enzym. Biol. Clin.* 7, 167–184.
- Schmidt, E., Schmidt, F. W., Herfarth, C., Opitz, K. & Vogell, W. (1966) *Enzym. Biol. Clin.* 7, 185–202.
- Schmidt, E., Schmidt, F. W., Both, R., Ohlendorf, S., Raupach, R. & Staar, U. (1983) Enzyme patterns in liver and serum of rats during growth and aging. In: *Progress in clinical enzymology* (Goldberg, D. & Werner, M., eds.). Vol. II. Masson, New York pp. 53–61.
- Schmidt, E. & Schmidt, F. W. (1960) *Klin. Wochenschr.* 38, 957–962.
- Schmidt, E. & Schmidt, F. W. (1962/63) *Enzym. Biol. Clin.* 2, 201–222.
- Schmidt, E. & Schmidt, F. W. (1979) Enzyme. Grundlagen der Enzymdiagnostik. In: *Wissenschaftliche Tabellen Geigy, Teilband Haematologie und Humangenetik*. Basel pp. 146–175.
- Keller, P. (1979) *Kleintier Praxis* 24, 51–68.
- Keller, P. (1981) *Am. J. Vet. Res.* 42, 575–582.
- Clampitt, R. B. & Hart, R. J. (1978) *J. Comp. Pathol.* 88, 607–621.
- Nagode, L. A., Frajola, W. J. & Loeb, W. F. (1966) *Am. J. Vet. Res.* 27, 1385–1393.
- Zinkl, J. G., Bush, R. M., Cornelius, C. E. & Freedland, R. A. (1971) *Res. Vet. Sci.* 12, 211–214.
- Pette, D. (1977) Enzymmuster des energieliefernden Stoffwechsels im Skelettmuskel. In: *Muskelstoffwechsel, körperliche Leistungsfähigkeit und Diabetes mellitus. Theorie und Praxis.* (Jahnke, K., Mehnert, H. & Reis, H. E., eds.). Schattauer Verlag, Stuttgart–New York pp. 21–46.
- Metzenauer, P. & Lutz, J. (1981) *Z. Versuchstierk.* 23, 94–101.
- Boyd, J. W. (1962) *Res. Vet. Sci.* 3, 256–268.
- Lindena, J., Friedel, R., Rapp, K., Sommerfeld, U., Trautschold, I. & Deerberg, F. (1980) *Mech. Ageing Dev.* 14, 379–407.
- Knox, W. E. (ed.) (1972) Enzyme patterns in fetal, adult and neoplastic rat tissues. Karger, Basel.
- The Universities Federation for Animal Welfare (UFAW) (eds.) (1968) Methoden zur tierschutzgerechten Tötung von Tieren. German translation by Gärtner, K. & Ruppert, E.
- Recommendations of the German Society for Clinical Chemistry (1972) Standardisation of methods for the estimation of enzyme activities in biological fluids. *This J.* 10, 281–291.
- Hollaar, L., Jansen, P. Y., van der Laarse, A., Dijkshoorn, N. J., Bogers, A. J. & Huysmans, H. A. (1984) *Clin. Chim. Acta* 139, 47–53.
- Würzburg, U., Hennrich, N., Lang, H., Prellwitz, W., Neumeier, D. & Knedel, M. (1976) *Klin. Wochenschr.* 54, 357–360.
- Hermens, W. Th., van der Laarse, A. & Witteveen, S. A. G. J. (1979) Enzymatic infarct sizing: Factors influencing the choice of the marker enzyme. In: *Enzymes in Cardiology: Diagnosis and Research* (Hearse, D. J. & de Leiris, J., eds.) Wiley, Chichester pp. 339–353.
- Lowry, O. H., Rosenbrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* 193, 265–275.
- Schmidt, E. & Schmidt, F. W. (1962/63) *Enzym. Biol. Clin.* 2, 223–232.
- Herzfeld, A. (1972) *Enzyme* 13, 246–251.
- Blum, H. E., Weber, B., Deus, B. & Gerok, W. (1981) The mitochondrial creatine kinase isoenzyme from human heart muscle. In: *Creatine kinase isoenzymes. Pathophysiology and clinical application* (Lang, H., ed.) Springer Verlag, Berlin pp. 19–30.
- Wevers, R. A., Reutelingsperger, C. P., Dam, B. & Soons, J. B. (1982) *Clin. Chim. Acta* 119, 209–223.
- Yasmineh, W. G., Ibrahim, G. A., Abbasnezhad, M. & Awad, E. A. (1978) *Clin. Chem.* 24, 1985–1989.
- McDaniel, H. G., Yeh, M., Jenkins, R., Freeman, B. & Simmons, J. (1984) *Am. J. Physiol.* 246, H483–H490.
- Idell-Wenger, J. A., Grotzyhann, L. W. & Neely, J. R. (1978) *J. Biol. Chem.* 253, 4310–4318.
- Rej, R. (1978) *Clin. Chem.* 24, 1971–1979.
- Lindena, J., Sommerfeld, U., Hoepfel, C., Wolkersdorfer, R. & Trautschold, I. (1983) *Enzyme* 29, 100–108.

45. Mühlhaus, K. (1981) Experimente zum Austrittsmechanismus von Zellenzymen am Modell des isoliert-perfundierten Rattenherzens. M. D. Thesis, Medizinische Hochschule Hannover.
46. Möhr, J. R., Mattenheimer, H., Holmes, A. W., Deinhardt, F. & Schmidt, F. W. (1971) *Enzyme* 12, 99–116.
47. Schmidt, E., Schmidt, F. W. & Möhr, J. (1967) *Clin. Chim. Acta* 15, 337–342.
48. Pappas, N. J. Jr. (1980) *Clin. Chim. Acta* 106, 223–229.
49. Mühlhaus, K. (1977) Isolierung und Charakterisierung von Isoenzymen der Malatdehydrogenase aus menschlicher Leber als Voraussetzung für ihre immunologische Differenzierung. Ph. D. Thesis, Universität Hannover.
50. Teschke, R., Neufeind, M., Nishimura, M. & Strohmeyer, G. (1983) *Gut* 24, 625–630.
51. Nishimura, M. & Teschke, R. (1982) *Biochem. Pharmacol.* 31, 377–381.
52. Schmidt, E., Schmidt, F. W., Chemnitz, G., Kubale, R. & Lobers, J. (1980) *Klin. Wochenschr.* 58, 709–718.
53. Sharov, V. G., Saks, V. A., Smirnov, V. N. & Chazov, E. I. (1977) *Biochim. Biophys. Acta* 468, 495–501.
54. Haralambie, G. (1978) *Enzyme* 23, 182–186.
55. Hintz, C. S., Lowry, C. V., Kaiser, K. K., McKee, D. & Lowry, O. H. (1980) *Am. J. Physiol.* 239, C58–C65.
56. Thorstensson, A., Elwin, K., Sjoedin, B. & Karlsson, J. (1976) *Scand. J. Clin. Lab. Invest.* 36 821–826.
57. Thorstensson, A., Sjoedin, B., Tesch, P. & Karlsson, J. (1977) *Acta Physiol. Scand.* 99, 225–229.
58. Prellwitz, W., Kapp, S., Neumeier, D., Knedel, M., Lang, H. & Heuinkel, D. (1978) *Klin. Wochenschr.* 56, 559–565.
59. Visser, M. P., Krill, M. T., Muijtens, A. M., Willems, G. M. & Hermens, W. T. (1981) *Clin. Chem.* 27, 1845–1850.
60. Sjoedin, B., Thorstensson, A., Frith, K. & Karlsson, J. (1976) *Acta Physiol. Scand.* 97, 150–157.
61. Font, B., Vial, C., Goldschmidt, D., Eichenberger, D. & Gautheron, D. C. (1981) *Arch. Biochem. Biophys.* 212, 195–203.
62. Vassault, A. (1983) Lactate dehydrogenase. In: *Methods of enzymatic analysis*. Third Edition. Vol. III (Bergmeyer, H. U., ed.) Verlag Chemie, Weinheim pp. 118–126.
63. Rej, R. (1984) *CRC Crit. Rev. Clin. Lab. Sci.* 21, 98–186.
64. Recommendations of the German Society for Clinical Chemistry. Standardisation of methods for the estimation of enzyme activities in biological fluids.
this J. 8, 659–660 (1970)
this J. 9, 464–465 (1971)
this J. 10, 281–291 (1972)
this J. 12, 392 (1974)
this J. 15, 255–260 (1977)
65. Bergmeyer, H. U., Hörder, M. & Moss, D. W. (1978) *Clin. Chem.* 24, 720–721.
66. Bergmeyer, H. U. & Hörder, M. (1980) *Clin. Chim. Acta* 105, 147F–172F.
67. Yasmin, W. G., Pyle, R. B., Hanson, N. Q. & Hultman, B. K. (1976) *Clin. Chem.* 22, 63–66.
68. Haralambie, G. & Berg, A. (1978) *Enzyme* 23, 104–107.
69. van der Laarse, A., Dijkshoorn, N. J., Hollaar, L. & Caspers, I. (1980) *Clin. Chim. Acta* 104, 381–391.
70. Urdal, P., Urdal, K. & Stromme, J. H. (1983) *Clin. Chem.* 29, 310–313.
71. Bendz, R., Stroem, S. & Olin, C. (1980) *Eur. J. Cardiol.* 12, 25–39.
72. Bendz, R. & Stroem, S. (1981) *Scand. J. Thorac. Cardiovasc.* 15, 199–204.
73. Neumeier, D., Prellwitz, W. & Knedel, M. (1981) Creatine kinase isoenzymes after myocardial infarction. In: *Creatine kinase isoenzymes* (Lang, H., ed.) Springer Verlag, Berlin pp. 132–156.
74. Haralambie, G. & Uy, J. (1980) *Clin. Cardiol.* 3, 192–196.
75. Herzfeld, D. A., Rosenoer, V. M. & Raper, S. M. (1976) *Pediatr. Res.* 10, 960–964.
76. Friedel, R., Bode, R., Trautschold, I. & Mattenheimer, H. (1976) this J. 14, 119–128.
77. Bär, U. & Ohlendorf, S. (1970) *Klin. Wochenschr.* 48, 776–780.
78. Diederichs, F., Mühlhaus, K., Wittenberg, H. & Trautschold, I. (1986) this J. 24, 3–9.
79. Inoue, M., Hori, M., Nishimoto, Y., Fukui, S., Abe, H., Wada, H. & Minamino, T. (1978) *Br. Heart J.* 40, 1251–1256.
80. Smith, A. F., Wong, P. C. P. & Oliver, M. F. (1977) *J. Mol. Med.* 2, 265–269.
81. Schmidt, E., Schmidt, F. W. & Otto, P. (1967) *Clin. Chim. Acta* 15, 283–289.
82. Willems, G. M., Lambi, F. H. H. & Hermens, W. Th. (1980) Analysis of simultaneously sampled plasma activities of aspartate aminotransferase, α -hydroxybutyrate dehydrogenase and creatine kinase in patients with acute myocardial infarction. In: *Advances in clinical cardiology*. Vol. I. (Kreuzer, H., Palmley, W. W., Rentrop, P. & Heiss, H. W., eds.) Gerhard Witzstrock Verlag pp. 482–493.
83. Guder, W. G., Habicht, A., Kleißl, J., Schmidt, U. & Wieland, O. H. (1975) this J. 13, 311–318.

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