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Catalytic Enzyme Activity Concentration in Plasma of Man, Sheep, Dog, Cat, Rabbit, Guinea Pig, Rat and Mouse

Approach to a Quantitative Diagnostic Enzymology, I. Communication

By J. Lindena and I. Trautschold †

Abteilung Klinische Biochemie, Medizinische Hochschule Hannover

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

Summary: For the most convenient laboratory animals in experimental medicine such as the sheep, dog, cat, rabbit, guinea pig, rat and mouse, the catalytic concentrations of seventeen enzymes and the protein and albumin concentrations in plasma were determined. The corresponding data for man were taken from the literature. The experimental conditions were selected so as to minimize the influence on catalytic activity, variance and statistical distribution pattern of such factors as handling procedures, the choice of blood drawing technique, the choice of anaesthetic agent for distinct species and the preparation procedure of plasma from the blood specimen. Because these considerations have been largely neglected to date in experimental enzymology, normal values for most enzymes require revision and were in some instances established for the first time in certain species. Our use of assay conditions, which were identical for all animal species, satisfies current requirements in clinical chemistry with respect to standardization and optimization of enzyme catalytic activity concentration measurement, and allowed comparison of these enzymes. The catalytic activities established this way will be the basis for a later comparative study of their kinetics of adjustment in plasma.

*Katalytische Konzentration von Enzymen im Plasma von Mensch und Versuchstieren
Versuch der Begründung einer quantitativen Diagnostischen Enzymologie, I.*

Zusammenfassung: Bei den in der experimentellen Medizin gebräuchlichen Versuchstieren wie Schaf, Hund, Katze, Kaninchen, Meerschweinchen, Ratte und Maus wurden im Plasma die katalytischen Konzentrationen von 17 Enzymen und die Protein- und Albuminkonzentration bestimmt. Die entsprechenden Werte für den Menschen wurden der Literatur entnommen. Die experimentellen Bedingungen waren so gewählt, daß eine Minimierung der die Höhe, Varianz und statistische Verteilungsform der Merkmale am stärksten belastenden Einflußgrößen wie die experimentelle Manipulation der Tiere, die jeweilige Art der Blutentnahmemethode, die bei bestimmten Spezies notwendige Anaesthesie und die Aufbereitung des Blutes zu Plasma gewährleistet erscheint. Weil diese Einflußgrößen in der experimentellen Enzymologie bisher weitgehend vernachlässigt wurden, mußten die Referenzwerte für viele Enzyme revidiert werden; sie wurden für andere bei bestimmten Spezies erstmals erstellt. Die für alle Spezies identischen analytischen Bedingungen entsprachen den gegenwärtigen Anforderungen der Klinischen Chemie an Standardisierung und Optimierung der Bestimmung von katalytischen Enzymaktivitätskonzentrationen und erlauben darüber eine vergleichende Betrachtung dieser Merkmale. Die so erstellten katalytischen Aktivitäten werden die Grundlage für eine vergleichende Studie ihrer Verlaufs-Kinetik im Plasma sein.

Introduction

The determination of cellular enzymes by catalytic activity or by plasma and serum concentration (1) are still the most frequently applied methods in clinical chemistry. Cellular enzymes in plasma serve as sensitive indicators for disturbances of the cellular integrity induced by pathological conditions and give information about the location, extent, and severity of the disturbances and their further development (2). Although clinical enzymology indubitably has its merits, its working basis is more or less empirical.

Our knowledge of pathophysiological mechanisms such as enzyme release, distribution, transport, and elimination, all of which affect the adjustment of catalytic concentration in the intravascular space, lacks behind improvements in the areas of analytical and instrumental development.

The simple question, however, of where the normal activity in plasma originates from, is still unanswered.

Every attempt at a more quantitative description of kinetics of enzyme activity course in the extracellular space has to start with an estimation of the normal catalytic enzyme activity in the intravascular space. Plasma, because it is easily obtainable is the most common research material in clinical enzymology.

Whereas reference values exist in man for the most frequently used enzymes, and are well standardized, such standardization is often lacking in the clinical chemistry of animal research.

Although the major requirements for blood drawing and preparation of samples for small laboratory animals and dogs have been recommended by *Friedel et al.* (3–5), these general rules are not usually followed. In fact, so-called normal values of catalytic enzyme activities in plasma, especially for the mouse, rat, guinea pig and rabbit are subject to some criticism. Comprehensive data for sheep are completely lacking and data for cats are incomplete. For dogs reference values have been established for only some enzymes (6, 7).

It is therefore necessary to establish reference values in plasma for the most frequently used enzymes in diagnostic enzymology in the most commonly used experimental laboratory animals. In the sheep, dog, cat, rabbit, guinea pig, rat and mouse these values were determined with special regard for possible interfering factors.

These included variations on blood sampling method, preparation of the sample, and metabolic changes secondary to handling (3, 4, 8–10). The values for man are taken from the literature.

Materials and Methods

Animals and blood drawing technique

Sheep

Unanaesthetized male animals (Schwarzköpfiges Fleischschaf) weighing 25–33 kg were used. They were delivered from an accredited breeding farm (Domäne Rodenberg).

An intravenous catheter (*Bardi—Cath*, Bard GB) was inserted into the external jugular vein at least two days prior to blood sampling.

Dogs

Blood was drawn from the cephalic vein of unanaesthetized male German shepherds. They weighed from 24 to 31 kg. A 20-gauge needle was used without venous occlusion.

Cats

Female cats weighing 2.3 to 3.4 kg were used. They were from a common mongrel strain of a local breeding department.

Blood was drawn through a 20-gauge needle from the external jugular vein after sedation with a subcutaneous injection of 25 mg/kg ketamine (Ketavet®).

Rabbits

Male conscious rabbits from a local strain ("Helle Großsilber") weighing 2.6 to 3.0 kg were used. Blood was withdrawn from the central ear artery with a 20-gauge needle.

Guinea pigs

Male animals from the inbred strain Pirbright White, weighing 550 to 750 g were used. Blood was withdrawn from the common carotid artery through a polyethylene catheter (Portex, Great Britain; i. d. 0.58 mm, o. d. 0.98 mm) after anaesthesia with an intraperitoneal injection of a mixture of xylazine (Rompun®, diluted with 0.15 mol/l NaCl to 1.7 g/l), and ketamine (Ketavet®, diluted to 10 g/l) at 16 and 100 mg/kg body weight, respectively.

Rats

Male rats from the inbred Lewis/Ztm strain, weighing 260 to 290 g and aged 90 to 110 days were used. The same anaesthesia was used as for guinea pigs described above. A description of the blood sampling technique from the common carotid artery has been given in detail (8) and the same principles hold true for the guinea pig.

Mice

Male mice from the inbred BALB/cAnN Han strain, weighing 27 to 32 g were used. Blood was drawn through a Silastic catheter (Dow Corning Corporation, USA; i. d. 0.64 mm, o. d. 1.19 mm) deeply inserted into the external jugular vein. Again anaesthesia was identical to that for guinea pigs described above.

Blood samples were drawn between 08.00 and 09.00 h. They were heparinized (~ 50 U/ml whole blood). Blood was centrifuged immediately for 2 min at 12 000 g at room temperature on an Eppendorf centrifuge 3200 (Netheler and Hinz, Hamburg). Plasma was portioned, kept on ice and frozen at -70 °C within one hour after blood drawing.

Housing conditions and diet of the animals

Sheep were held under open-air and stable conditions at the local animal facility and received hay, oats and pellets (Raiffeisen, Hannover).

Dogs lived in an animal room ($17 \pm 1^\circ\text{C}$; $60 \pm 5\%$ relative humidity, light period from 06.00–18.00 h) in cages of 1.5×1.5 m on gratings. Feeding was once a day in the afternoon with Doko (Trouw, Thedinghausen).

Cats lived in animal rooms behind a barrier ($22 \pm 1^\circ\text{C}$, 60% relative humidity, light period from 06.00–18.00 h). Feeding was ad libitum with Latz (Quaker Latz, Euskirchen) and additionally with milk.

Guinea pigs, rats and mice were kept in polycarbonate cages ("Typ III Makrolon") in groups of 2, 4 and 15, respectively; rabbits in single cages of Nirosta. The light period was 07.00–19.00 h, temperature $22 \pm 1^\circ\text{C}$, and relative humidity was 55%. Water was supplied from polycarbonate bottles. Feeding was ad libitum with Altromin 1324 (Altromin, Lage) for rats and mice, and pellets (Eggersman, Rinteln) for rabbits (120 g once a day) and guinea pigs (ad libitum). Guinea pigs, rats and mice were free from all the pathogens specified in the GV-SOLAS list (11).

It must be emphasized that animals which underwent blood drawing by catheter techniques (guinea pigs, rats and mice) were not sacrificed for plasma preparation purposes only. They also were used for the investigation of catalytic activities in various blood cells (12–14), and other parenchymal tissues (15). Blood from rabbits, dogs and sheep was obtained from animals which were held at the local animal facility for various experimental purposes. Blood was drawn prior to any other experimental work. The cats were for breeding purposes only.

Man: The data for catalytic activity in the plasma of man were taken from the cited literature. Only those data were used, that were obtained by comparable analytical methods.

Determination of catalytic enzyme activity concentration; protein and albumin concentration; quality control

Enzyme assays were carried out at 25°C .

Aldolase, acid phosphatase and prostatic acid phosphatase were assayed at 37°C and the aldolase catalytic activity was corrected to 25°C with a factor of 0.41.

Measurements were performed in duplicate. Sample and reagent blanks were also run.

Enzyme catalytic activity was not calculated if the change of absorbance was smaller than 0.002/min.

Protein was determined with the standard biuret procedure; albumin with the bromocresol green method (27).

Internal quality control was assured by pooled dog plasma samples, which had been stored at -70°C . Each group of five specimens was accompanied by one pool sample. Thereby the long-term precision expressed as coefficient of variation (%) was as follows:

lactate dehydrogenase 6.5,
malate dehydrogenase 5.8,
aspartate aminotransferase 6.8,
alanine aminotransferase 5.3,
creatine kinase 4.1,
adenylate kinase 9.3,
cholinesterase 5.0,
alkaline phosphatase 6.6,
leucine arylamidase 6.6,
aldolase 6.3,
phosphohexose isomerase 4.0.

The data are expressed as the mean and include quality control measurements from l. c. (14, 15, 28), in which catalytic activities were determined in lymph, tissue and erythrocytes. Daily quality control for the other enzymes was established with commercial control sera of varying batch number.

Results

Enzyme catalytic concentrations and mass concentration of protein and albumin for man and animals are summarized in table 1.

Enzyme	EC No.	Reference	Test manufacturer
Lactate dehydrogenase	1.1.1.27	l. c. (16)	Boehringer Mannheim
Malate dehydrogenase	1.1.1.37	Bergmeyer & Bernt (17)	Boehringer Mannheim
Isocitrate dehydrogenase	1.1.1.42	Wolfson et al. (18)	Boehringer Mannheim
Glutamate dehydrogenase	1.4.1.3	l. c. (16)	Boehringer Mannheim
γ -Glutamyl transferase	2.3.2.2	Substrate: <i>L</i> - γ -glutamyl-3-carboxy-4-nitroanilide Persijn & van der Slik (19), Szasz et al. (20)	Boehringer Mannheim
Aspartate aminotransferase	2.6.1.1	l. c. (16)	Boehringer Mannheim
Alanine aminotransferase	2.6.1.2	l. c. (16)	Boehringer Mannheim
Creatine kinase	2.7.3.2	l. c. (16) with the addition of EDTA (21)	Boehringer Mannheim
Adenylate kinase	2.7.4.3	Friedel et al. (3)	Boehringer Mannheim
Cholinesterase	3.1.1.8	Prellwitz et al. (22) Substrate: butyrylthiocholine iodide	self prepared
Alkaline phosphatase	3.1.3.1	l. c. (16)	Merck, Darmstadt
Acid phosphatase	3.1.3.2	Andersch & Szczypinski (23), Substrate: <i>p</i> -nitrophenyl phosphate	Behring, Marburg
α -Amylase	3.2.1.1	Schiwara (24), Substrate: maltoheptaose modified	Boehringer Mannheim
Amino acid arylamidase	3.4.11.2	l. c. (16)	Boehringer Mannheim
Aldolase	4.1.2.13	Beisenherz et al. (25)	Boehringer Mannheim
Phosphohexose isomerase	5.3.1.9	Bueding & MacKinnon (26)	Behring, Marburg

Tab. 1. Enzyme catalytic activity concentrations, protein and albumin concentration in plasma of man, sheep, dog, cat, rabbit, guinea pig, rat and mouse. Catalytic and mass concentrations are given in U/l and g/l. Values are the mean with standard error of the mean (SEM). Number of determinations in brackets. The data for man were taken from the cited authors. Human data are from male adults (>30 years). — = not determined < = below the detection limit

	Man	Sheep	Dog	Cat	Rabbit	Guinea pig	Rat	Mouse
Lactate dehydrogenase	179 ± 0.79 (2255)	705 ± 29.3 (16)	25.4 ± 1.23 (49)	52.3 ± 2.4 (23)	36.3 ± 1.7 (25)	70.8 ± 5.70 (14)	41.1 ± 1.22 (36)	130.7 ± 7.11 (18)
Malate dehydrogenase	71 ± 1.81 (88)	325 ± 19.5 (16)	43.0 ± 2.84 (36)	66.6 ± 2.2 (23)	31.4 ± 12.7 (32)	196 ± 5.78 (19)	25.7 ± 0.83 (38)	98.3 ± 3.78 (20)
Isocitrate dehydrogenase	2.0 (44)	2.56 ± 0.52 (14)	2.25 ± 0.24 (20)	3.4 ± 0.2 (20)	35.0 ± 2.41 (37)	37.8 ± 3.29 (17)	<	<
Glutamate dehydrogenase	4 (362)	12.5 ± 1.69 (14)	<	<	6.39 ± 0.59 (37)	4.87 ± 0.59 (15)	2.37 ± 0.08 (60)	—
γ-Glutamyl transferase	6 — 28	39.1 ± 3.0 (16)	2.17 ± 0.06 (62)	1.2 ± 0.1 (20)	3.78 ± 0.19 (38)	3.18 ± 0.18 (19)	<	<
Aspartate aminotransferase	11.1 (708)	51.5 ± 2.6 (15)	10.1 ± 0.51 (37)	11.1 ± 0.8 (17)	17.2 ± 1.23 (34)	25.1 ± 1.87 (18)	29.9 ± 3.98 (53)	18.2 ± 0.57 (19)
Alanine aminotransferase	11.1 (395)	9.37 ± 0.59 (16)	18.3 ± 0.81 (29)	21.3 ± 1.2 (23)	37.8 ± 1.75 (35)	28.0 ± 1.38 (20)	23.4 ± 0.40 (59)	19.4 ± 0.53 (19)
Creatine kinase	≤80 (386)	31.2 ± 2.1 (14)	22.2 ± 1.20 (38)	39.4 ± 2.1 (20)	91.4 ± 4.76 (25)	88.8 ± 3.80 (17)	79.8 ± 2.03 (34)	37.9 ± 1.79 (20)
Adenylate kinase	—	25.8 ± 1.89 (16)	20.1 ± 1.01 (20)	12.8 ± 0.7 (17)	20.2 ± 1.25 (33)	24.5 ± 1.13 (20)	28.6 ± 0.72 (40)	—
Cholinesterase	4740 ± 89.1 (726)	32.8 ± 2.88 (16)	2927 ± 164 (37)	975 ± 31.9 (23)	87.8 ± 5.79 (29)	4418 ± 195 (19)	81.6 ± 1.27 (57)	1776 ± 35.9 (19)
Alkaline phosphatase	115 (708)	91.4 ± 3.67 (16)	21.3 ± 1.2 (37)	109 ± 6.1 (25)	78.0 ± 4.34 (31)	281 ± 12.2 (20)	597 ± 12.2 (62)	152 ± 3.66 (19)
Acid phosphatase	6.02 ± 0.36 (22)	11.1 ± 1.52 (15)	—	1.5 ± 0.1 (22)	23.9 ± 1.90 (38)	42.0 ± 2.86 (17)	24.6 ± 0.45 (59)	11.0 ± 1.14 (12)
Prostatic acid phosphatase	0.40 (9)	—	—	—	1.90 ± 0.27 (38)	3.10 ± 0.37 (15)	13.0 ± 0.39 (59)	1.03 ± 0.12 (12)
α-Amylase	68.8 (194)	74.5 ± 7.13 (16)	1537 ± 92.8 (36)	1340 ± 54.4 (25)	315 ± 19.8 (36)	2459 ± 103 (18)	2696 ± 81.9 (40)	2150 ± 62.1 (20)
Amino acid arylamidase	25.5 ± 0.19 (1586)	14.9 ± 1.0 (16)	10.5 ± 0.67 (35)	2.9 ± 0.1 (25)	34.2 ± 1.16 (36)	300 ± 7.75 (20)	25.9 ± 0.34 (62)	14.8 ± 0.26 (20)
Aldolase	1.70 ± 0.06 (33)	124 ± 9.80 (16)	7.36 ± 0.35 (35)	10.8 ± 0.8 (24)	17.1 ± 1.22 (37)	13.7 ± 0.72 (18)	15.5 ± 0.45 (54)	12.5 ± 0.56 (19)
Phosphohexose isomerase	39.1 ± 0.84 (208)	360 ± 26.1 (15)	53.6 ± 2.72 (41)	443 ± 14.8 (25)	237 ± 7.23 (33)	1021 ± 27.3 (20)	50.0 ± 1.08 (39)	87.1 ± 2.25 (19)
Protein	71.0 ± 0.33 (111)	83.0 ± 2.40 (14)	55.2 ± 2.65 (37)	69.4 ± 2.1 (24)	65.5 ± 1.53 (36)	71.4 ± 1.28 (22)	70.8 ± 1.18 (42)	55.7 ± 1.11 (20)
Albumin	46.3 ± 0.63 (30)	41.6 ± 2.60 (14)	29.3 ± 1.70 (37)	39.7 ± 0.8 (24)	34.3 ± 1.10 (36)	50.3 ± 1.13 (23)	34.9 ± 0.62 (41)	29.1 ± 0.90 (20)

Markedly higher catalytic concentrations compared with other species were found for lactate dehydrogenase, malate dehydrogenase, glutamate dehydrogenase and γ -glutamyl transferase in sheep, for amino acid arylamidase and phosphohexose isomerase in guinea pigs and for alkaline phosphatase and prostatic acid phosphatase in rats; markedly lower catalytic concentrations were seen for α -amylase and aldolase in man, for cholinesterase and α -amylase in sheep, for alkaline phosphatase in dogs, for acid phosphatase and amino acid arylamidase in cats and for malate dehydrogenase in rats.

Discussion

When determining catalytic activities in animal blood samples several influences which directly affect the value and variability of the catalytic activities have in the past been forgotten or overlooked.

1. Metabolic disturbances due to blood drawing or anaesthesia

Because of the lack of an easily accessible and suitable site for blood drawing in mice, guinea pigs and rats, and in order to avoid pain, it is necessary to anaesthetize these animals for blood drawing. Ether or pentobarbital often cause tremendous muscular activity which leads to an uncontrolled and highly accelerated lymphatic inflow with fairly high enzyme content (28, 41).

After application of a combination of xylazine and ketamine, however, the animals quietly fall asleep (42–44).

Although highly increased plasma values for enzymes such as creatine kinase after intramuscular injection of drugs are well known, the intramuscular mode of anaesthesia in small laboratory animals has no influence on the level of catalytic activities in plasma if blood samples are drawn within about 5 min after injection. Lymphatic transport of enzymes occurs slowly immediately after anaesthetics are given, and ceases when the animal is fully anaesthetized.

To avoid any risk whatsoever, it is advisable to inject anaesthetics intraperitoneally. Within about 3 min the animals have reached the tolerance stage.

An unavoidable stress reaction is nevertheless caused even by touching the animal's cage. Packed cell volume and protein are altered within 2 min after moving the cage and heart rate is increased. Significant alterations of enzyme activities were not seen after application of this particular stress (9).

In rabbits, pigs and sheep, changes in protein and enzyme levels were demonstrated in the course of restraining procedure (10, 45, 46). Emotional stress for dogs and cats is highly reduced if familiar attendants draw blood. Nevertheless, for cats a sedative or even an anaesthetic is recommended.

2. Blood drawing techniques

In dogs, cats and rabbits, peripheral veins or arteries are easily accessible. For long term observations in these animals chronic jugular vein catheter techniques similar to those recommended for sheep (10) can be applied to reduce emotional stress.

In guinea pigs the most convenient method is puncture of the retroorbital venous plexus. In rats and mice decapitation or aortic incision, heart puncture or tail vein puncture (rat only) have all been used. All these techniques, however, are afflicted with several disadvantages as to their effects on the level, variability and statistical distribution pattern of catalytic activities in the blood. Decapitation, heart puncture and aortic incision cause injury to muscle tissue. Enzymes like lactate dehydrogenase, malate dehydrogenase, aspartate aminotransferase, creatine kinase, adenylate kinase and phosphohexose isomerase (from here on referred to as "critical" enzymes) are found in striated and heart muscle with many times the catalytic activity of that seen in blood (from 1×10^3 to 2×10^5 fold) and are in fact, as determined from the cited investigations, falsely increased in blood after use of these methods in rabbits (47), guinea pigs (48, 49), rats (2, 8, 50) and mice (3, 51, 52).

This conclusion can be drawn from direct comparison of values from heart puncture, aortic incision etc. vs. those derived from catheter techniques (3, 8).

Cannulation of an artery or vein is the most reliable method for blood drawing in experimental enzymology, because only minimal blunt dissection of tissue is required. A practical long-term catheter in rats is lacking.

The most convenient technique in small laboratory animals is puncture of the retroorbital plexus. *Friedel et al.* (3), however, demonstrated, that beside contamination with interstitial fluid and damaged tissue, obstruction of venous back flow caused the non-filtrable plasma elements (e.g. protein) to become more concentrated owing to increased hydrostatic pressure. This phenomenon also holds true for tourniquet application in man (53). The "critical" enzymes especially are falsely elevated in plexus blood from guinea pigs (47) and rats (3, 47, 54–56). An

exception to this rule are the values of *Keller* (57) for retroorbital blood from guinea pigs and rats, which agree with our data. Blood from the tail veins of rats also seems unsuitable because the "normal" values are much too high. This technique also yields only small volumes of blood (58). Enzymes of mainly visceral origin like alanine aminotransferase, cholinesterase, alkaline phosphatase etc., show markedly lower catalytic activities in muscle tissue (up to 3×10^2 fold higher compared to plasma) than the "critical" enzymes. Invasive techniques for blood sampling, which are accompanied by soft tissue and muscle trauma, contribute very little therefore to the observed catalytic activity changes of these enzymes in blood.

3. Use of plasma or serum for catalytic activity determination

For all laboratory animals, including dogs, it holds true that catalytic activities, especially of glycolytic enzymes, increase in serum with time during blood clotting. These enzymes are primarily released from platelets (4, 5, 54). Use of serum instead of plasma therefore results in falsely increased activity as is obvious in dogs (5, 47, 54, 59), rabbits (5, 47, 59–61), guinea pigs (4, 47, 48, 59), rats (5, 47, 54–56, 59) and mice (4, 59). *Metzenauer & Lutz* (52) heparinized guinea pigs and rats before catheter blood drawing. The heparin doses of 60 U per animal, however, seems too low to prevent in vitro enzyme release from platelets, as concluded from high aspartate aminotransferase values. The high values of γ -glutamyl transferase in rat serum (62, 63) may also be due to its release from platelets during clotting (5).

Considering further the up to 60-fold higher catalytic activities of creatine kinase in platelets of small laboratory animals and dogs compared with man (12, 13), it is highly probable that the high values for creatine kinase in serum of these animals originate from platelets during clotting (61, 64, 65). A platelet-derived acid phosphatase is seen in serum but not in plasma (66).

To prevent clotting, *Friedel et al.* (4, 5) recommended serum preparation from native plasma, i. e. immediate centrifugation of whole blood without any anticoagulants and removal of the fibrin clot prior to measuring. This procedure, however, involves the risk of platelet-mediated clotting and release of enzymes in animals like rats and mice with a very fast clotting system. We therefore prefer the preparation of plasma from whole blood with a small amount of heparin in the syringe (~ 50 U/ml whole blood). This small amount does not interfere with subsequent analysis.

In heparinized whole blood of the rat no enzyme activity increase was observed within the first hour after withdrawal. Thereafter an increase of lactate dehydrogenase, malate dehydrogenase and aspartate aminotransferase was found, which was quite small in comparison to the increase observed in clotted blood (5, 54).

In man and dogs, however, these enzymes did not change when heparinized blood was allowed to stand for up to 4 h (54).

We prefer immediate and high speed centrifugation of heparinized blood (12 000 g, 2 min). Centrifugation speed and time should be chosen to provide a platelet free plasma, in as much as the assay is performed under conditions that result in lysis of platelets (67–69).

4. Haemolysis

Lactate dehydrogenase reflects the degree of haemolysis by its increased plasma catalytic activity better than other enzymes. The lowest haemoglobin concentration consistently categorized by the technologist as "trace haemolysis" is about 0.3 g/l (4, 70) and gave rise to lactate dehydrogenase of 23 U/l and to aspartate aminotransferase of 2 U/l in man. These increases agree quite well with theoretical ones, calculated for these as well as other enzymes, from the enzyme content of erythrocytes and the appropriate plasma distribution space of man and animals (12, 13).

5. Statistical distribution pattern of enzymes in plasma

An indication of the validity of our data on catalytic enzyme activity determination in the plasma of animals are our lower values for the mean as well as for variance as compared with previous results. This especially holds true for "critical" enzymes of small laboratory animals in comparison with previous "normal" values. The previously described alterations in catalytic activity in plasma were caused by the experimental methods. This experimentally induced variance far exceeds any biological and analytical changes and, in contrast to the latter ones, is not randomly distributed. Neglect of the factors outlined above results in falsely elevated catalytic activities of cellular enzymes in plasma and increases its variability by decreasing the slope of the *Gaussian* curve, i. e. by a shift of the distribution curve to the right.

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This means that all experimental influences can only increase the catalytic activities in plasma, as was evident from a positive skew for these data points. In contrast, catheter blood samples, which were almost free from experimentally induced variance, were normally distributed (8).

The necessity to transform catalytic activity data for statistical analysis is probably due to changes inherent in the experimental procedure (54, 65).

6. Diurnal variation; age and sex

On selected enzymes of diagnostic importance *Dabew* et al. (58) found diurnal variation in neither rabbit nor rat plasma, whereas *Statland* et al. (71) in man demonstrated a significant variation with time for acid phosphatase only.

Definite sex and age associations for several enzymes are well known in man (survey: l. c. (72)). For alkaline phosphatase, age-related changes have also been reported in animals (54, 55, 73–76). These changes have a physiological explanation related to bone growth development. Empirically observed age and sex-related changes in several enzymes have been measured in rat plasma (73, 76), and were reviewed up to 1973 for mammals as a whole (77). A most remarkable sex difference for cholinesterase in rat plasma has been reported by *Schmidt & Schmidt* (78).

7. Optimized assay conditions

Application of analytical methods developed for human serum (16) to the plasma of various animals does not necessarily result in an optimisation for each animal species. Isoenzymes possess distinct kinetic properties and different isoenzyme proportions may be masked or amplified by a standardized assay.

The use of optimized tests or commercially available tests has been used widely in plasma and also in human and animal tissues. In veterinary medicine reliable reference values, especially for dogs have been established with these "optimized" assays (6, 7, 79).

Comprehensive data on plasma catalytic concentrations in one or more common laboratory animals which meet the requirements outlined above are available in very few cases (6–8, 57, 79). Other studies, especially many published in the last few years, have neglected one or more of these points and have therefore resulted in erroneous data (52, 54, 56, 58–60, 80).

Any approach to a quantitative description of the dynamic state of enzymes in the extracellular space, which is the general aim of this series of investigations, must rely on reliable data of catalytic concentrations in plasma.

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Joachim Lindena, DVM
Abteilung Klinische Biochemie
Medizinische Hochschule Hannover
Konstanty-Gutschow-Straße 8
D-3000 Hannover 61