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STABILITY OF SOME SERUM ENZYMES IN SHEEP, CATTLE, AND SWINE DURING STORAGE AT DIFFERENT TEMPERATURES

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The stability of enzyme activity in serum or plasma is of considerable practical importance in routine analysis for clinical purposes as well as in research work on these diagnostic criteria.

Many investigations on the stability in vitro have been undertaken, but the results are widely divergent. Most of them show great instability of enzyme activity, whereas some authors report a remarkable high stability. Differences in species, treatment of the blood before analysis, analytical methods etc. may account for the variations in results.

Most of the work in this field has been performed on human sera. With the great differences existing in normal serum activity and in isoenzyme pattern between species, however, it is not advisable uncritically to draw parallels from one species to another.

In veterinary medicine blood or serum samples often have to be sent or transported over long distances to a clinical laboratory. Knowledge about the keeping qualities of the enzymes, and the way the samples can best be handled and stored before analysis, is necessary if considerable errors of the enzyme interpretation should be avoided.

The purpose of the present investigation was to examine by

routine methods under various temperature conditions the stability of four serum enzymes which have been shown to be of value in the diagnosis of heart, liver, and skeletal muscle injuries: aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, and α-hydroxybutyrate dehydrogenase*).

MATERIAL AND METHODS

Normal, healthy animals were used for storage testing of the serum enzymes: 16 (14) dairy cows, 15 adult ewes, and 16 pigs of about 4 months.

Blood samples were collected from the jugular vein in cattle and sheep and from the anterior vena cava in pigs. The blood was allowed to clot and serum was obtained by immediate centrifuging for 10—15 min. at 3,000 r.p.m.

All sera were analysed on the sampling day and stored at three different temperatures, at room temperature (22°C), in the refrigerator (4°C), and in the deep-freezer (—20°C). Sera stored at room temperature and in the refrigerator were analysed for 5 successive days. Samples from the deep-freezer were reanalysed only once, after 38, 36, and 32 days, respectively, in cattle, sheep, and swine. Before analysis, samples from the refrigerator and the deep-freezer were kept at room temperature until serum held 20—22°C. After the quantity needed had been pipetted off, the samples were replaced in the refrigerator.

Analytical procedures were according to Sigma Technical Bulletins. For aspartate aminotransferase and alanine aminotransferase: Sigma Technical Bulletin No. 505, 1964. Sigma-Frankel (S-F) units. For lactate dehydrogenase: Sigma Technical Bulletin No. 500, 1960. Berger-Broida (B-B) units. For α-hydroxybutyrate dehydrogenase: Sigma Technical Bulletin No. 495, 1964. Sigma units.

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*) The nomenclature for enzymes suggested in the *Report of the Commission on Enzymes* (1961) is used in this paper. Abbrevations are not approved by the Commission. For practical reasons abbreviations introduced by other authors in accordance with the new systematic and trivial names are used in tables and figures. Aspartate aminotransferase (EC 2.6.1.1), AspAT, previously known as glutamate-oxalacetate-transaminase (GOT). Alanine aminotransferase (EC 2.6.1.2), AlAT, earlier named glutamate-pyruvate-transaminase (GPT). Lactate dehydrogenase (EC 1.1.1.27), LDH. For α-hydroxybutyrate dehydrogenase (HBD) no classification number is given.

RESULTS

The results are given in Tables 1 and 2 and in Figs. 1—4. In Table 1 are given the mean serum values and standard deviations of all four enzymes investigated in the three species during storage at different temperatures. Table 2 shows the results of analyses of covariance for the samples stored at room and refrigerator temperatures and the variance analyses for the frozen sera. Figs. 1—4 show the linear regression on storage time for the four enzymes in the different animal sera stored at room and refrigerator temperatures. (The use of linear regression does not necessarily imply that the true regression is linear).

As seen from Table 2, a significant difference between animals of the same species existed for all enzymes investigated.

The effect of storing conditions on serum activity varied greatly with animal species and enzymes.

Aspartate aminotransferase

In all animal species a small but significant rise of the aspartate aminotransferase activity occurred when serum was stored at room temperature.

In cattle no significant change in activity occurred when serum was stored in the refrigerator for 5 days or when frozen for 38 days. Swine serum also kept very well in the deep-freezer, whereas a significant drop was observed when stored in the refrigerator. In sheep a significant decrease occurred during storage both in the refrigerator and in the deep-freezer.

Alanine aminotransferase

A significant increase of the enzyme activity was observed in sera from cattle and swine stored at room temperature. For cattle a similar rise also occurred in the cold. Sheep serum was fairly stable under all storing conditions. For swine serum a highly significant loss was observed after storage both in the refrigerator and in the deep-freezer.

Lactate dehydrogenase

In cattle total lactate dehydrogenase activity was stable during storage at room temperature and when frozen. In the refrigerator a highly significant loss was observed. In sheep serum a significant decrease was found under all storing con-

Table 1. Mean serum activities ($\bar{x}$) and standard deviations (s) of AspAT, AlAT, LDH and HBD in cattle, sheep, and swine during 5 days of storage at room temperature (22°C), in refrigerator (4°C), and after 32—38 days of deep-freezing (—20°C).

	Animal species	Temp. °C	1 $\bar{x}$	1 s	2 $\bar{x}$	2 s	3 $\bar{x}$	3 s	4 $\bar{x}$	4 s	5 $\bar{x}$	5 s	32—38 $\bar{x}$	32—38 s
AspAT, S—F units	cattle	22	70.6	10.9	70.4	11.3	74.2	11.7	71.8	11.5	76.1	11.3		
		4	70.6	10.9	72.6	11.3	72.9	10.9	71.3	10.2	70.3	11.3		
		—20	69.3	10.2[1]									71.8	10.0
	sheep	22	93.5	15.8	92.3	15.7	94.5	16.5	93.8	15.0	100.5	17.8		
		4	93.5	15.8	95.2	17.2	90.0	15.5	89.5	15.2	87.5	14.1		
		—20	96.3	22.6									77.5	17.1
	swine	22	40.1	16.7	40.1	16.7	38.9	16.6	42.7	17.2	42.7	16.4		
		4	40.1	16.7	39.9	16.7	39.9	17.4	39.0	17.0	38.8	17.3		
		—20	40.1	16.7									40.1	11.7
AlAT, S—F units	cattle	22	20.4	2.3	21.1	2.4	22.6	2.3	22.4	2.8	22.1	3.0		
		4	20.4	2.3	21.4	2.4	22.9	2.8	22.4	2.9	21.8	2.5		
		—20	20.8	2.9									19.6	3.0
	sheep	22	15.3	2.9	16.1	2.4	13.5	3.2	14.1	3.3	18.3	4.7		
		4	15.3	2.9	16.5	3.8	15.6	3.8	16.3	3.4	15.6	3.5		
		—20	12.2	2.8									11.2	2.2
	swine	22	25.4	4.0	25.1	4.1	23.8	3.8	25.1	4.1	27.1	3.9		
		4	25.4	4.0	25.3	4.0	23.3	3.6	22.8	4.0	22.4	3.5		
		—20	25.4	4.0									21.8	3.3
LDH, B—B units	cattle	22	1606	140	1656	136	1595	112	1593	107	1638	95		
		4	1606	140	1609	124	1611	124	1449	59	1414	81		
		—20	1606	140									1604	139
	sheep	22	951	107	921	114	919	106	909	109	879	102		
		4	951	107	916	110	951	119	920	133	885	113		
		—20	951	107									887	111
	swine	22	991	188	886	171	811	162	761	163	767	161		
		4	991	188	736	149	643	129	620	140	553	122		
		—20	991	188									973	191
HBD, Sigma units	cattle	22	506	59	505	48	486	66	446	52	438	49		
		4	506	59	489	61	435	53	425	51	428	50		
		—20	506	59									402	55
	sheep	22	296	46	282	51	258	45	257	51	249	43		
		4	296	46	297	51	274	53	266	50	251	45		
		—20	296	46									252	39
	swine	22	408	105	286	82	236	61	236	69	251	73		
		4	408	105	221	59	182	44	183	43	184	46		
		—20	408	105									361	104

[1]) Serum for deep-freezing from cattle and sheep originated from other animals than those used for room and refrigerator temperature tests. The same sera were also used for all tests of LDH and HBD in these animals.

Table 2. Results from analysis of covariance for samples stored at room and refrigerator temperatures and analysis of variance for frozen sera, means ($\bar{x}$), and error coefficients of variation (C.V.).

Enzyme	Animal species	Storage temp. °C	Days n	Animals n	Mean squares and significance: between animals	linear regression on storage time[1])	error	$\bar{x}$	C.V.
		22	5	16	562***	238**	22.1	72.6	6.5
	cattle	4	5	16	584***	6.0	4.6	71.5	3.0
		—20	2	14	130	44	74.0	71.8	12.0
		22	5	15	1184***	365**	33.9	94.9	6.1
AspAT	sheep	4	5	15	1189***	472***	8.5	91.1	3.2
		—20	2	15	768***	2670***	32.4	77.5	7.4
		22	5	16	1385***	101***	5.7	40.9	5.8
	swine	4	5	16	1442***	20**	1.9	39.5	3.5
		—20	2	14	359***	0	57.5	40.1	19.0
		22	5	16	26.3***	34.2***	1.9	21.7	6.4
	cattle	4	5	16	32.5***	21.0***	1.0	21.8	4.6
		—20	2	14	16.9***	9.0**	0.6	19.6	4.0
		22	5	15	43.1***	24.0	6.6	15.5	16.6
AlAT	sheep	4	5	15	55.6***	0.17	1.5	15.9	7.7
		—20	2	15	12.3***	7.0*	0.8	11.2	7.9
		22	5	16	57.7***	18.9**	5.8	25.3	9.5
	swine	4	5	16	67.4***	117***	1.5	23.8	5.1
		—20	2	16	23.7***	105***	2.7	21.8	7.5
		22	5	14	59490***	1.61	3521	1617.4	3.7
	cattle	4	5	14	48249***	413114***	5055	1537.8	4.6
		—20	2	14	33265**	10	5557	1604.3	4.6
		22	5	15	50271***	36504***	1854	915.6	4.7
LDH	sheep	4	5	15	63471***	24194***	1453	924.5	4.1
		—20	2	15	21935***	30083***	1724	887.3	4.7
		22	5	16	138391***	526129***	2373	843.2	5.8
	swine	4	5	16	101849**	1579069***	6244	708.6	11.1
		—20	2	16	70928***	2450	618	973.4	2.6
		22	5	14	12043***	52458***	831	476.0	6.1
	cattle	4	5	14	11382***	66534***	1135	456.5	7.4
		—20	2	14	5112*	75712***	1383	401.6	9.3
		22	5	15	9017***	21313***	531	268.5	8.6
HBD	sheep	4	5	15	10609***	21865***	383	276.9	7.1
		—20	2	15	2735*	15053***	835	251.5	11.5
		22	5	16	28662**	213087***	2703	283.5	18.3
	swine	4	5	16	16737	379276***	4589	235.9	28.7
		—20	2	16	19720***	18288*	2311	360.6	13.3

[1]) For frozen samples (—20) the mean squares between sampling day and 32—38th day are placed in this column.
*** indicates $P < 0.001$. ** indicates $P < 0.01$. * indicates $P < 0.05$.

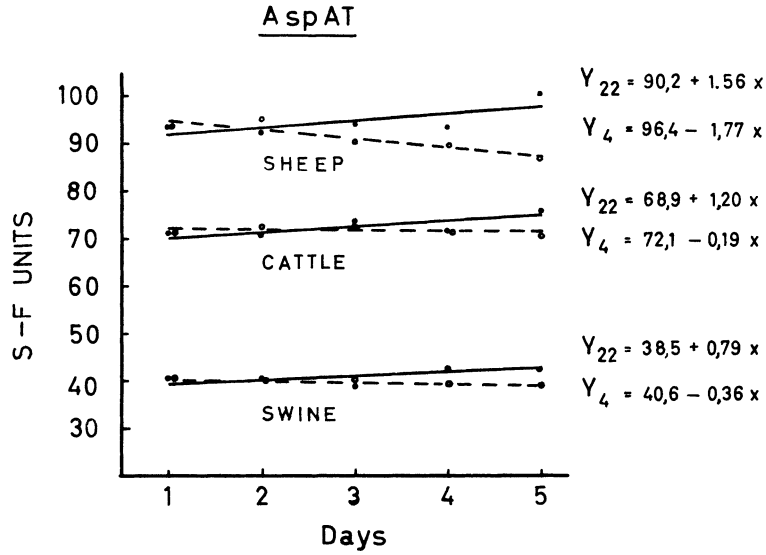

Figure 1. Regression lines and equations of aspartate aminotransferase on storage time at room temperature (Y_{22}—••) and in the refrigerator (Y_4--oo).

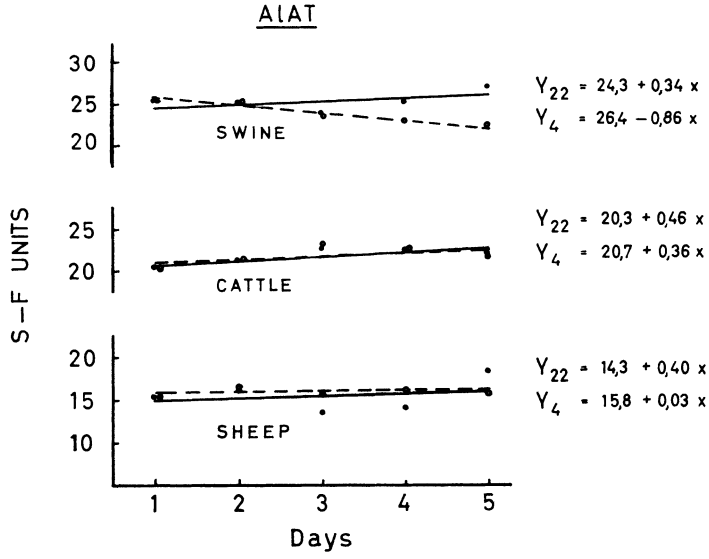

Figure 2. Regression lines and equations of alanine aminotransferase on storage time at room temperature (Y_{22}—••) and in the refrigerator (Y_4--oo).

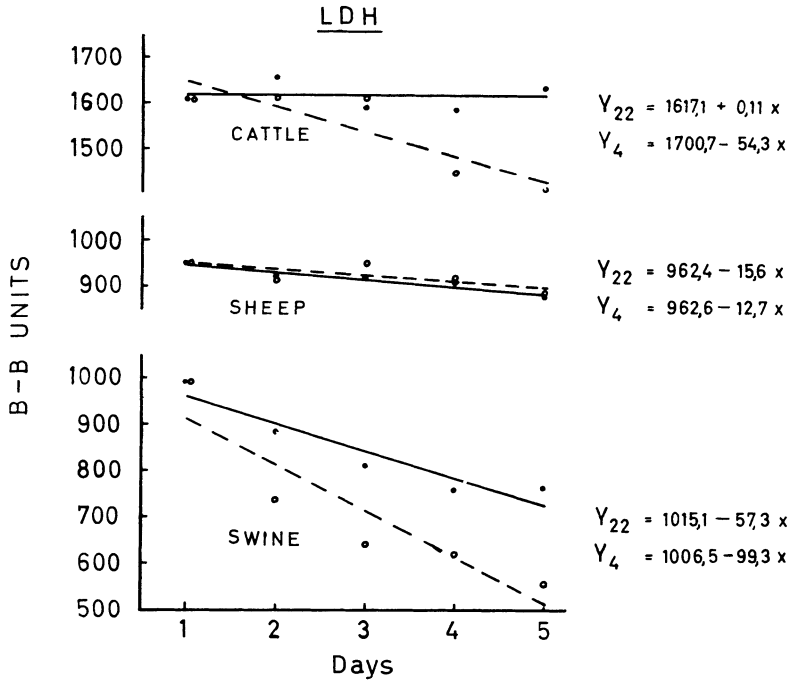

Figure 3. Regression lines and equations of lactate dehydrogenase on storage time at room temperature (Y_{22}—••) and in the refrigerator (Y_4--oo).

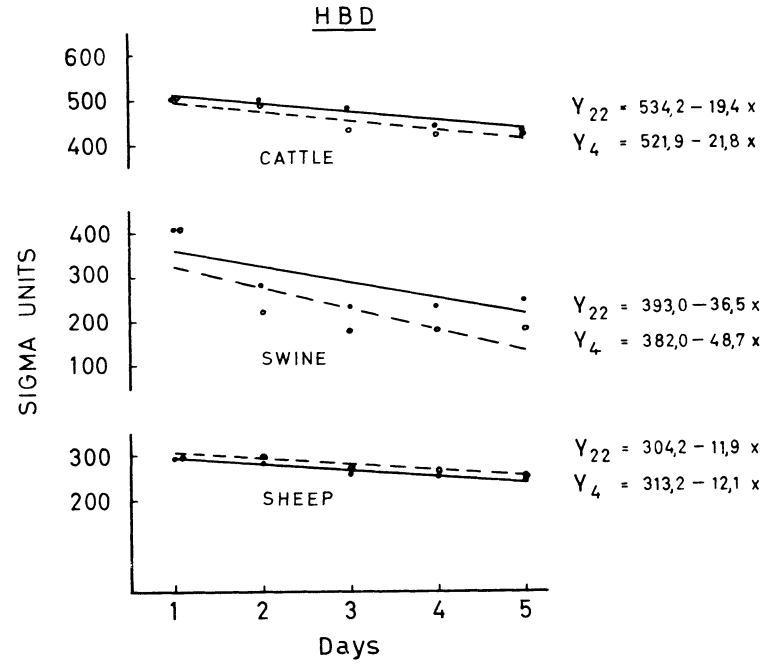

Figure 4. Regression lines and equations of α-hydroxybutyrate dehydrogenase on storage time at room temperature (Y_{22}—••) and in the refrigerator (Y_4--oo).

ditions. Swine serum kept very well in the deep-freezer, but showed highly significant losses at room temperature and even greater in the refrigerator. After one day at 4°C the activity decrease was 25.7 % of the initial value, and after 5 days 55.8 %.

α-hydroxybutyrate dehydrogenase

Significant losses of this enzyme were found in sera from all species under all storing temperatures, most pronounced in swine serum kept in the refrigerator.

DISCUSSION

The initial serum enzyme levels presented in Table 1 are in good agreement with what is found in normal animals by other investigators and discussed in earlier reports (*Tollersrud & Ribe* 1967, and *Tollersrud & Nafstad,* in press).

When considering the presented keeping qualities of the serum enzymes it must be emphasized that even if changes in activity during storage were found to be highly statistically significant, they are not all of the same importance for diagnostic use.

Errors of practical consequence would thus hardly occur in transferase activities if serum were kept under similar conditions as those of the experiment. The changes which were found during the storage were less than the observed standard deviations between animals.

An increase of aspartate aminotransferase activity in serum at room temperature has previously been observed in human medicine (*Feissli et al.* 1966). The cause is not known. In the present investigation the serum samples became more or less opaque, and the activity increase could possibly be explained by a bacterial growth. A significant increase was nevertheless found in the alanine aminotransferase activity of cattle serum kept in the refrigerator, where no discoloration of the sera could be seen.

The studies of lactate dehydrogenase confirmed earlier investigations and showed that this enzyme can be less stable at refrigerator than at room temperature. *Südhof & Wötzel* (1960) observed a rapid decline in human serum. Nearly 40 % of the initial activity was lost when human serum was stored for 48 hrs. at room or refrigerator temperatures, and about 30 % after

storage in the deep-freezer. Later publications have stated that human lactate dehydrogenase is sensitive to cold environments, and that the loss of activity is less pronounced at room temperature (*Kreutzer & Fennis* 1964, *Amelung et al.* 1966). *Feissli et al.*, however, did not agree with these findings since their results showed that human lactate dehydrogenase as well as aspartate and alanine aminotransferase had a high stability at 25°C and 4°C. A simulated transport by shaking serum samples in a water bath at 25°C did not affect their enzyme results for at least 3 days. A high stability of human serum enzymes was also found by *Schmidt & Schmidt* (1963).

Hyldgaard-Jensen (1966) reported a considerable fall in total lactate dehydrogenase activity in swine plasma after only 6—10 hrs. when serum was stored at 2—4°C. At room temperature the activity was stable for 4—5 days, and at —20°C for at least 2 months.

In the present study pig serum kept at room temperature showed a loss of activity of 22.6 % in the course of 5 days. Significant differences in plasma and serum total activity have not been reported, and the varying results may be due to the difference in the analytical methods used.

Lactate dehydrogenase seemed to be better kept by deep-freezing than when stored in the refrigerator. This was the case with cattle and swine. *Raabo* (1963) found that serum from cattle could be stored frozen without loss of activity for at least 6 months.

The value of α-hydroxybutyrate dehydrogenase determinations in veterinary medicine has not yet been established, and very little is found in the literature on this topic. In human medicine determinations of this component have proved to be of importance in the differential diagnostic work of heart and liver injuries. In some feeding experiments at this institute (not published) very close correlations are found between serum elevations of aspartate aminotransferase, total lactate dehydrogenase, and α-hydroxyburyrate dehydrogenase.

The activity loss of α-hydroxybutyrate dehydrogenase in swine serum stored in the refrigerator was even greater than that of lactate dehydrogenase; 45.8 % of the total activity was lost after 1 day. Stored at room temperature the loss was 29.9 %. In sheep and cattle the greatest decrease occurred during deep-freezing.

It is noticeable that α-hydroxybutyrate dehydrogenase, which has been considered to be strongly related to, if not synonymous with, the fast-moving anodic isoenzyme LDH_1 or LDH_2 (*Wilkinson* 1965), in animals is so sensitive to low temperatures. According to *Kreutzer & Fennis,* these "heart fractions" in human serum are the most stable isoenzymes of lactate dehydrogenase in the cold. They are also found to be more resistant to high temperatures than the slow-moving cathodic "liver fractions" LDH_4 and LDH_5 (*Wilkinson*).

All sera used in this investigation originated from healthy animals with enzyme values within normal ranges. How far the activity in sera with increased values due to pathologic conditions would show the same stability properties is not dealt with. In human serum *Amelung et al.* found that the percentage decrease in lactate dehydrogenase activity was independent of the initial value.

CONCLUSION

As a practical consequence of the stability tests presented, the following recommendations can be put forward:

If only transferases should be analysed in the sera, and the analysis for some reason cannot be performed on newly drawn blood, it is not very critical if the sera are stored unfrozen for up to 5 days. Owing to the risk of bacterial growth the sera should not be kept at room temperature for more than 3 days. When storage for a longer period is needed, deep-freezing may be used without too great losses.

In cases where lactate dehydrogenase and α-hydroxybutyrate dehydrogenase determinations are of interest, special account must be taken of the blood donor species. Pig serum has to be analysed as soon as possible after sampling, or frozen down promptly.

As a general rule, when all four enzymes are examined routinely in series of samples, and the work will require more than 1 day, the dehydrogenases should be analysed first followed by the transferases.

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SUMMARY

Owing to the increased use of serum enzyme determinations in veterinary diagnostic work, greater knowledge about the keeping qualities of different animal sera under various storing conditions seems desirable.

The present paper deals with the stability of serum aspartate aminotransferase (AspAT = GOT), alanine aminotransferase (AlAT = GPT), lactate dehydrogenase (LDH), and α-hydroxybutyrate dehydrogenase (HBD) in cattle, sheep, and swine.

Sera from 14—16 animals of each species were analysed daily for 5 days after storage at room temperature (22°C) and in the refrigerator (4°C). Samples kept in the deep-freezer (—20°C) were reanalysed once after 32—38 days.

Significant differences of serum activity were found between individuals for all enzymes in the three species.

Great variations were found in the stability of enzyme activities of different species.

To summarize, it may be said that the changes of transferase activities were less pronounced under the different storing conditions than those of the dehydrogenases investigated. Pig serum in particular showed heavy losses of the latter enzymes already after 1 day, more pronounced at refrigerator than at room temperature.

As a consequence of the results obtained, practical recommendations for analytical work on these enzymes are suggested.

ZUSAMMENFASSUNG

Die Stabilität einiger Serumenzyme bei Schaf, Rind und Schwein nach Aufbewahrung bei verschiedenen Temperaturen.

Die steigende Anwendung von Serumenzymbestimmungen in der tierärztlichen Diagnostik macht eine gute Kenntnis zur Stabilität der aktuellen Enzyme bei verschiedenen Tierarten unter variierenden Aufbewahrungsverhältnissen des Serums notwendig.

Die Untersuchungen, die hier vorgelegt werden, behandeln die Stabilität von Aspartataminotransferase (AspAT = GOT), Alaninaminotransferase (AlAT), Laktatdehydrogenase (LDH) und α-Hydroxybutyratdehydrogenase (HBD) bei Rind, Schaf und Schwein.

Serum von 14—16 Tieren jeder Tiergattung wurde an fünf nacheinander folgenden Tagen nach Aufbewahrung bei Zimmertemperatur (22°C) und im Kühlschrank (4°C) analysiert. Analysen von Proben, die im Gefrierschrank (—20°C) aufbewahrt worden waren, wurden nach dem Verlauf von 32—38 Tagen einmal wiederholt. Bei sämtlichen untersuchten Enzymen wurde ein signifikanter Unterschied in der Serumaktivität zwischen den Individuen der verschiedenen Tiergattungen festgestellt.

Weiter wurden grosse Variationen in der Stabilität der verschiedenen Enzyme bei den verschiedenen Tiergattungen festgestellt.

Summarisch kann gesagt werden, dass die Transferasen sich unter den unterschiedlichen Lagerungsverhältnissen besser hielten als die untersuchten Dehydrogenasen. Besonders zeigte Schweineserum schon nach dem Verlauf von 24 Stunden einen starken Fall in der Laktat- und α-Hydroxybutyratdehydrogenase. Der Rückgang war stärker markiert nach Lagerung im Kühlschrank als bei Zimmertemperatur.

Verhältnisse von praktischer Bedeutung zur best möglichen Bewertung dieser Enzyme für diagnostische Zwecke werden angedeutet.

SAMMENDRAG

Stabiliteten av noen serumenzymer hos sau, storfe og gris under oppbevaring ved forskjellig temperatur.

Økt bruk av serumenzymbestemmelser i veterinær diagnostikk nødvendiggjør et godt kjennskap til stabiliteten av de aktuelle enzymer hos forskjellige dyrearter under ulike oppbevaringsforhold av serum.

De undersøkelser som her presenteres, omhandler stabiliteten av aspartataminotransferase (AspAT = GOT), alaninaminotransferase (AlAT), laktatdehydrogenase (LDH) og α-hydroksybutyratdehydrogenase (HBD) hos storfe, sau og gris.

Serum fra 14—16 dyr av hver dyreart ble analysert 5 dager på rad etter oppbevaring ved værelsestemperatur (22°C) og i kjøleskap (4°C). Analyse av prøver oppbevart i fryseboks (—20°C) ble gjentatt en gang etter 32—38 dager. En signifikant forskjell i serumaktivitet mellom individer av de ulike dyreslag ble funnet for alle de undersøkte enzymene.

Det ble videre funnet store variasjoner i stabiliteten av de forskjellige enzymer hos de ulike dyrearter.

Summarisk kan en si at transferasene holdt seg bedre under de forskjellige lagringsforhold enn de undersøkte dehydrogenasene. Særlig griseserum viste stor nedgang i laktat- og α-hydroksybutyratdehydrogenase allerede etter 1 døgn, og nedgangen var mer markert etter lagring i kjøleskap enn ved værelsestemperatur.

Forhold av praktisk betydning for en best mulig vurdering av disse enzymer i diagnostisk øyemed er antydet.

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