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HEAT STABILITY OF SERUM LACTATE DEHYDROGENASE AND ITS ISOENZYMES IN YOUNG AND ADULT CATTLE AND SHEEP

EVALUATION OF A RELATIVE HEAT STABILITY TEST AND SERUM DETERMINATION OF α-HYDROXY-BUTYRATE DEHYDROGENASE IN DIAGNOSTIC WORK

By

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It is generally accepted that lactate dehydrogenase (LDH, L-lactate: NAD oxidoreductase, EC 1.1.1.27) isoenzymes leak out of a damaged tissue, causing changes in the serum isoenzyme pattern. In this way the damaged tissue superimposes its LDH pattern upon that of the serum. In myocardial infarction in man, the electrophoretically fast-moving LDH isoenzymes in serum are increased, because they are the predominant isoenzymes of human heart muscle. In acute hepatitis in man, the slowest moving isoenzyme increases in serum since it constitutes the dominant fraction of liver LDH.

A relative heat stability test of LDH has been introduced in the diagnosis of heart and liver diseases in man. The simplified fractionation of LDH isoenzymes is based upon the finding of *Wróblewski & Gregory* (1961) and *Strandjord et al.* (1962) that LDH isoenzymes which migrate electrophoretically faster towards the anode are relatively thermostable, whereas the slow-moving cathodic fractions are more heat labile.

The LDH activity which remains after incubation of a serum at 65 °C for 30 min. has been defined as "heat stable LDH", while the activity lost at 57 °C should represent the "heat labile LDH".

Normal levels have not yet been clearly defined, but *Wróblewski & Gregory* state that normal human serum contains 20—40 % heat stable LDH and 10—30 % of the heat labile fraction.

Rosalki & Wilkinson (1960) and *Elliot & Wilkinson* (1961) showed that the heart fraction of LDH had a greater affinity to α-ketobutyric acid than to pyruvic acid. When the former substrate was used they called the enzyme α-hydroxybutyrate dehydrogenase (HBD).

Determination of HBD in serum has been found valuable in human clinical diagnosis. It has been maintained by several authors that HBD is a more specific heart enzyme than LDH, and a close correlation has been found between HBD and the "heat stable LDH" ($r = 0.93$, *Blörstad* 1966). *Rosalki & Wilkinson* (1964) have maintained that HBD might replace the laborious separation of the organ specific LDH isoenzymes.

In previous experiments in this department (*Baustad & Tollersrud* 1969) the stability, including thermostability, of LDH and its isoenzymes in serum from normal pigs has been investigated by electrophoresis of fresh and heat-treated sera. In addition the correlation between LDH and HBD in the sera was studied.

The present paper deals with the same properties of enzymes in serum from cattle and sheep of different ages.

In order to obtain information about the usefulness of a relative heat stability test in the diagnostic work on cattle and sheep, the LDH isoenzyme patterns of some main tissues from these species have been investigated.

MATERIAL AND METHODS

Animals

The experimental animals consisted of eight fattening bull calves at an age of three and a half months and four adult cows, all of the Norwegian Red Cattle breed. Furthermore, eight 10 days old suckling lambs and six adult non-pregnant ewes of the native Norwegian short-tailed breed were used.

Blood was taken by venepuncture and serum obtained through immediate centrifugation for 15 min. at 3,000 r.p.m.

Enzyme analyses

Total LDH was analysed by the colorimetric method of *Berger & Broida* (1967).

HBD was analysed as outlined in Sigma tentative Tech. Bull. No. 495 (1964).

The electrophoretic separation of LDH isoenzymes was principally performed as earlier described for swine serum (*Baustad & Tollersrud* 1969). The buffer and gels, however, were modified according to recently published results of *Hyldgaard-Jensen et al.* (1968). As it has been found that veronal selectively inhibits LDH isoenzymes, and that citric acid is a specific activator of LDH_5, the buffer system was changed to a veronal-tris-citric acid buffer. Pyrophosphate was used instead of cyanide as a stimulator of colour formation, resulting in a minimum of background staining. Agarose was used in the colour agar layer, as the presence of colloids stimulates the colour development. Ionagar No. 2 (Oxoid) gave in our experiments a better separation than did Special Agar-Noble (Difco) used by the authors referred to.

Composition of buffer and gels:

Buffer: 0.042 M diethyl barbiturate (veronal) — 0.1 M tris — 0.021 M citric acid; the mixture was adjusted to pH 8.4. Agar gel layer: 1.0 % Ionagar No. 2 (Oxoid) in 0.035 M veronal, 0.0732 M tris, 0.0175 M citric acid and 0.0245 M-HCl (pH 8.2). Colour agar layer: 0.35 % agarose (L'Industrie Biologique Française S.A.) in 0.084 M tris + 0.025 M citric acid + 0.015 M pyrophosphate (pH 8.0).

The staining solution containing lactate, NAD, MTT-tetrazolium and phenazine methosulphate was composed as previously described by *Baustad & Tollersrud.*

The electrophoresis was run for 120 min. at 250 v and 30—35 mamp. (LDH isoenzymes in serum from cattle and sheep migrate at a slower rate than those from pigs). After incubation and fixation, the slides were kept in distilled water for only 24 hrs.

The soft agarose layer was then carefully removed under running water. The remaining coloured Ionagar layer was dried at 37°C overnight. Thus the duration of the whole procedure was reduced from 5—6 to 2 days.

For isoenzyme determination of tissues the supernatant of a 5 % homogenate was used. The tissue was thoroughly rinsed with cold saline and dried with filter paper. A quantity of 300 mg was suspended in 5.7 ml of tris-HCl buffer (0.1 M, pH 8.5) and homogenized with an Ultra-Turrax homogenizer for 60 sec. at a rate of 14,000 r.p.m. The homogenate was centrifuged for 10 min. at 38,000 × g.

For electrophoresis, the supernatant was diluted with swine serum in which LDH had been inactivated by heating at 65°C for 30 min. This was found to give a better separation than by addition of bovine albumin. The electrophoresis was run for 100 min. at 250 v and 30—35 mamp.

Heat treatment

One ml of undiluted serum of each sample was incubated at different temperatures in tubes sealed with parafilm in exactly con-

trolled water baths for 30 min. Aliquots of the heat treated sera were used for total LDH and HBD determinations and for electrophoresis.

The results obtained from serum without preincubation are recorded in the tables and figures under 37°C, since this is the working temperature in the routine analytic method used.

All determinations were made in duplicates.

RESULTS

Cattle

Calves. The results of LDH isoenzyme determinations in serum exposed to different temperatures are given in Table 1. From the total LDH values, the activity of the different isoenzymes are calculated and given in Table 2.

Cows. The LDH isoenzyme distribution in serum subjected to different temperatures is shown in Table 3. Calculated activity of the different isoenzymes is given in Table 4.

From Tables 2 and 4 and from Fig. 1 it is seen that 25—30 % of the total LDH activity in serum from cattle was lost after preincubation at 57°C for 30 min., and that only 2—3 % was left after heating to 65°C. A higher percentage of the anodic isoenzymes in cows than in calves caused a slightly inferior decrease of total LDH in adults at the lowest incubating temperatures.

Table 1. Percentage distribution and standard deviation (±s) of LDH isoenzymes in serum of calves.

Temperature	LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
37°C	39.0 ± 1.0	35.5 ± 1.5	19.7 ± 1.4	4.6 ± 1.4	1.2 ± 0.6
57°C	61.6 ± 2.5	31.4 ± 1.9	7.0 ± 0.7	0	0
60°C	90.3 ± 0.9	9.7 ± 0.9	0	0	0
63°	100	0	0	0	0
65°C	100	0	0	0	0

Table 2. Total LDH, calculated LDH activity of the isoenzymes, and HBD ±s in serum of calves.

Temperature	Total LDH	LDH_1	LDH_2	LDH_3	LDH_4	LDH_5	HBD
37°C	1831 ± 57	715 ± 70	651 ± 67	361 ± 40	83 ± 24	21 ± 12	600 ± 68
57°C	1272 ± 188	782 ± 112	400 ± 69	90 ± 17	0	0	370 ± 39
60°C	750 ± 121	677 ± 110	73 ± 12	0	0	0	170 ± 25
63°C	250 ± 45	250 ± 45	0	0	0	0	55 ± 17
65°C	51 ± 22	51 ± 22	0	0	0	0	0

Table 3. Percentage distribution ±s of LDH isoenzymes in serum of cows.

Temperature	LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
37°C	45.2 ± 2.1	29.1 ± 1.4	17.1 ± 1.5	7.6 ± 2.1	1.0 ± 0.2
57°C	65.7 ± 2.8	26.9 ± 2.2	7.4 ± 1.8	0	0
60°C	94.0 ± 2.9	6.0 ± 2.9	0	0	0
63°C	100	0	0	0	0
65°C	100	0	0	0	0

Table 4. Total LDH, calculated LDH activity of the isoenzymes, and HBD ±s in serum of cows.

Temperature	Total LDH	LDH_1	LDH_2	LDH_3	LDH_4	LDH_5	HBD
37°C	1765 ± 274	799 ± 135	515 ± 90	302 ± 57	132 ± 39	17 ± 2	491 ± 63
57°C	1303 ± 258	860 ± 201	350 ± 67	93 ± 13	0	0	393 ± 57
60°C	828 ± 172	777 ± 156	51 ± 27	0	0	0	231 ± 50
63°C	345 ± 53	345 ± 53	0	0	0	0	87 ± 23
65°	55 ± 0	55	0	0	0	0	0

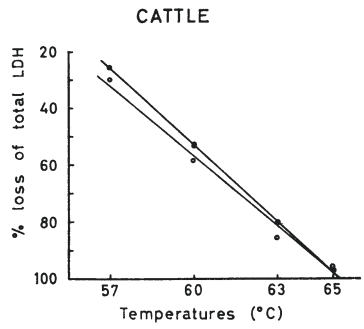

Figure 1. Loss of total LDH activity in serum of cattle (y) at preincubation temperatures in the range 57°C to 65°C (x).
Calves ooo: y = 8.31x—440.5
Cows ●●●: y = 8.95x—483.7

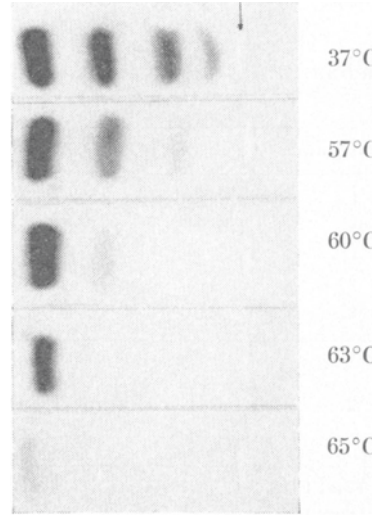

Figure 2. LDH isoenzymes in serum from cattle after preincubation at different temperatures. The arrow indicates point of application. The anode to the left.

The zymograms of Fig. 2 illustrate the thermostability of the different LDH isoenzymes in serum of cattle (calf). The cathodic isoenzymes, LDH_4 and LDH_5, were lost after heating to 57°C, and the intermediate forms, LDH_2 and LDH_3 were practically inactivated at 60°C. LDH_1 retained nearly all its original activity by heating to 60°C, whereafter a more rapid decline occurred to 65°C.

Sheep

Serum of sheep was treated in the same way as described for cattle, though other incubating temperatures had to be chosen.

Lambs. LDH isoenzyme distribution in serum of young lambs is given in Table 5. Calculated LDH activity of the different isoenzymes is given in Table 6.

Ewes. LDH isoenzyme distribution in serum of adult sheep is shown in Table 7. Calculated enzyme activity of the different isoenzymes is given in Table 8.

LDH in sheep serum was more sensitive to heat than that of serum from cattle. The enzyme furthermore proved to be less thermostable in serum from lambs than from adult sheep, probably owing to a higher percentage of the intermediate and cathodic fractions. The same phenomenon was to some extent observed in cattle.

Table 5. Percentage distribution ±s of LDH isoenzymes in serum of lambs.

Temperature	LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
37°C	29.5 ± 3.5	16.6 ± 1.9	35.8 ± 2.3	18.1 ± 5.7	0
50°C	38.5 ± 3.3	16.8 ± 1.9	34.9 ± 3.2	9.8 ± 4.7	0
53°C	63.2 ± 4.8	11.0 ± 2.5	25.8 ± 3.7	0	0
57°C	86.6 ± 7.0	10.6 ± 3.2	2.8 ± 4.1	0	0
60°C	0	0	0	0	0

Table 6. Total LDH, calculated LDH activity of the isoenzymes, and HBD ±s in serum of lambs.

Temperature	Total LDH	LDH_1	LDH_2	LDH_3	LDH_4	LDH_5	HBD
37°C	1452 ± 350	425 ± 99	241 ± 67	518 ± 127	268 ± 109	0	365 ± 120
50°C	1021 ± 280	396 ± 128	171 ± 48	359 ± 109	95 ± 34	0	226 ± 84
53°C	708 ± 263	451 ± 191	78 ± 35	178 ± 50	0	0	96 ± 40
57°C	237 ± 145	204 ± 127	25 ± 17	8 ± 16	0	0	0
60°C	0	0	0	0	0	0	0

Table 7. Percentage distribution ±s of LDH isoenzymes in serum from ewes.

Temperature	LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
37°C	48.4 ± 4.4	10.6 ± 1.1	31.1 ± 7.5	8.9 ± 6.8	1.0 ± 0.8
50°C	61.5 ± 5.7	7.8 ± 1.7	28.7 ± 4.6	2.0 ± 2.5	0
53°C	71.7 ± 5.4	4.9 ± 2.8	23.4 ± 5.4	0	0
57°C	91.0 ± 4.5	9.0 ± 2.5	0	0	0
60°C	100	0	0	0	0

Table 8. Total LDH, calculated LDH activity of the isoenzymes, and HBD ±s in serum from ewes.

Temperature	Total LDH	LDH_1	LDH_2	LDH_3	LDH_4	LDH_5	HBD
37°C	758 ± 121	365 ± 60	80 ± 7	235 ± 66	70 ± 52	8 ± 8	209 ± 20
50°C	575 ± 64	353 ± 50	45 ± 14	165 ± 33	12 ± 14	0	168 ± 20
53°C	475 ± 39	340 ± 36	24 ± 14	111 ± 24	0	0	142 ± 13
57°C	218 ± 36	198 ± 26	20 ± 9	0	0	0	56 ± 18
60°C	38 ± 15	38 ± 15	0	0	0	0	0

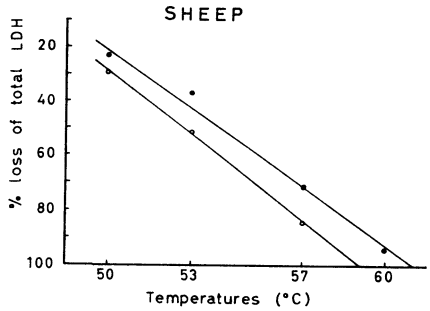

Figure 3. Loss of total LDH activity in serum of sheep (y) at preincubation temperatures in the range 50°C to 60°C (x).
Lambs ooo: y = 7.76x—358.6
Ewes ●●●: y = 7.29x—344.3

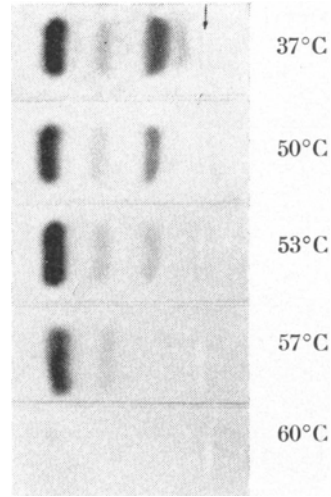

Figure 4. LDH isoenzymes in serum from sheep after preincubation at different temperatures.

As seen from Fig. 3, 70 % of total LDH was lost in serum of adult sheep after heating to 57°C, whereas 84 % was inactivated in serum from lambs. Although young animals showed about twice as high LDH activity as that found in serum from adults, total inactivation occurred at a lower temperature in lambs than in ewes.

The zymograms of Fig. 4 illustrate the thermostability of the different isoenzymes in serum from adult sheep. The most heat-labile fractions, LDH_5 and LDH_4 were totally lost at 53°C. Unlike that found in man, swine, and cattle, sheep serum is richer in LDH_3 than in LDH_2. LDH_2, however, was more thermostable than LDH_3. LDH_1 showed no loss of significance when heated to 53°C. About one-half of its calculated original activity had disappeared at 57°C, and only 10 % was left at 60°C.

Correlation between LDH and HBD

In Tabel 9 correlation coefficients are given between total LDH and HBD as well as between the "heat stable" isoenzymes LDH_1, LDH_2, and HBD. The material includes values of untreated sera and of samples preincubated at temperatures giving electrophoretic zones both of LDH_1 and LDH_2.

Table 9. Correlation coefficients between LDH and HBD in serum from young and adult cattle and sheep.

		Calves	Cows	Lambs	Ewes
Total LDH /HBD	r =	0.97	0.91	0.95	0.96
LDH_1/HBD	r =	0.25	0.34	0.38	0.50
LDH_2/HBD	r =	0.96	0.95	0.94	0.92

As previously found in swine serum (*Baustad & Tollersrud* 1969) a statistical significant correlation ($P < 0.05$) existed between total LDH and HBD in sera from cattle and sheep, whereas the correlation between the most heat stable LDH isoenzyme, LDH_1, and HBD was not significant. Significant correlations were, on the other hand, found between LDH_2 and HBD.

LDH isoenzyme distribution in some tissues from calves and sheep

The value of a serum LDH isoenzyme determination as a diagnostic aid is assumed to be dependent on the isoenzyme

distribution in the tissue(s) affected. If the isoenzyme pattern in main organs such as liver, heart, kidney, and skeletal muscles is nearly identical, a serum LDH fractionation will be of limited diagnostic value in diseases of these organs.

The zymograms of Figs. 5 and 6 show the obtained LDH isoenzyme pattern in organs from adult sheep and calves.

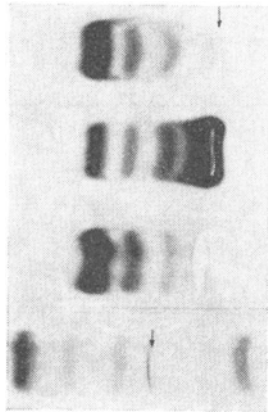

Figure 5. LDH isoenzyme distribution in organs from sheep.

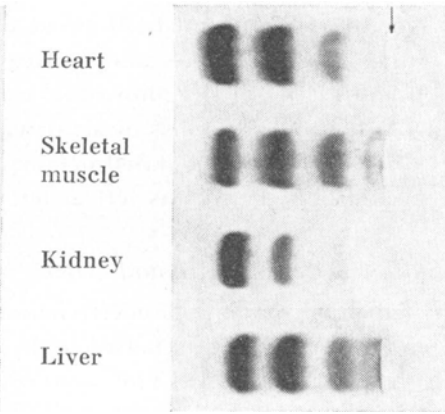

Figure 6. LDH isoenzyme distribution in organs from calf.

As seen from Fig. 5, distinct differences exist in the normal isoenzyme distribution in organs of sheep. The most striking difference was observed between skeletal muscle and the other tissues investigated. Whereas the anodic and intermediate isoenzymes were prevalent in extracts of heart, kidney, and liver, skeletal muscle (M. gracilis) showed greater activity of the slow-moving fractions LDH_4 and LDH_5.

It should be noted that in liver of adult sheep an LDH_5 fraction with exceptionally high cathodic electrophoretic mobility could be demonstrated (Fig. 5).

In contrast to what was found in sheep, the isoenzyme distribution in the same organs from calves did not show such characteristic differences. Both heart, skeletal muscle, liver, and kidney were dominated by the anodic and intermediate isoenzymes, though the percentage activity varied between organs, as seen in Table 10.

These results indicate that serum LDH isoenzyme determinations, or a relative heat stability test, might theoretically be use-

Table 10. Percentage distribution of LDH isoenzymes (1, 2, 3, 4, 5) in tissues from calf and adult sheep.

	Calf					Sheep				
	1	2	3	4	5	1	2	3	4	5
Heart	42.1	37.9	17.3	2.4	0.3	64.8	17.3	13.5	3.9	0.5
Skeletal m.	28.0	36.1	25.9	9.8	0.2	21.0	9.0	17.3	37.8	14.9
Kidney	59.4	27.3	7.0	6.0	0.3	68.5	24.0	6.3	0.8	0.4
Liver	33.9	37.9	17.5	9.9	0.8	57.3	11.2	11.2	6.5	13.8

ful in the diagnostic work on certain diseases of sheep. In cattle, on the other hand, it is more doubtful if an LDH fractionation has any diagnostic value.

DISCUSSION

The present investigation confirms results reported previously (*Tollersrud* 1969) that a marked difference exists in serum activity of total lactate dehydrogenase and α-hydroxybutyrate dehydrogenase between adult cattle and sheep. In contrast to what has been found in very young pigs and calves, lambs show a high serum activity of LDH compared to adult animals (*Tollersrud & Baustad* 1970).

The percentage isoenzyme distribution in adult sheep serum is in accordance with the results of *Paulson et al.* (1966). In tissues of lambs these authors, as well as *Boyd* (1964) found a still more marked cathodic predominance of the skeletal muscle isoenzymes than described here. A very high LDH_5 fraction has also been found in skeletal muscle of swine (*Hyldgaard-Jensen & Jensen* 1967).

The experiments have further shown that a distinct difference exists in the thermostability of LDH in serum from cattle and sheep. The heat resistance of LDH in animal sera seems to be considerably lower than in human serum. The results clearly indicate that attention must be paid to the species concerned if a relative heat stability test were used as an aid in veterinary diagnosis. Procedures worked out for human clinical purposes may give misleading results when applied directly to animal sera.

It must, however, be taken into consideration that LDH heat stability can be influenced by other factors, such as addition of its coenzym, $NADH_2$ (*Wróblewski & Gregory* 1961). After exposure to 65°C *Paunier & Rotthauwe* (1963) found the remaining

activity in human serum to be twice as high when $NADH_2$ had been added to serum as in samples without.

Human serum has to be diluted before heating to 65°C to avoid coagulation. The animal sera investigated did not coagulate at this temperature. Phosphate buffer has been recommended as a diluent (*Berger & Broida* 1967). However, phosphate buffer might change the thermostability. In our experiments serum dilutions with phosphate buffer caused a decrease of 10—15 % in total LDH when compared to dilution with distilled water.

Increased values of total LDH are found in several disease states. One of the most widespread diseases in lambs and young sheep in Norway is nutritional muscular dystrophy. Under these conditions slow-moving, heat-labile LDH isoenzymes would be expected to be released from the skeletal muscles to the blood stream causing increased total LDH values. As the fractions LDH_4 and LDH_5 in sheep serum are inactivated by heating to 50°—53°C for 30 min., a considerable fall in total LDH compared to non-preincubated serum will be expected in cases of muscular dystrophy. Further experiments, however, are needed to confirm the reliability of a heat stability test in cases of muscular dystrophy, and to define more clearly the limit between normal and pathologic values. In normal sheep serum 20—30 % of total LDH is inactivated by heating to 50°C for 30 min. In cases of acute muscular dystrophy this loss will probably be considerably higher, provided muscular isoenzymes are proportionally released to the blood. In animals with increased levels of LDH due to lesions in other organs than skeletal muscles, the percentage decrease at this temperature will obviously be lower. Thus sheep fed herring meal causing highly increased serum LDH activity, mainly LDH_1, without clinical or pathologic changes showed less than 10 % loss at 50°C (*Tollersrud,* in press).

Increased serum levels of other enzymes such as aspartate aminotransferase and alanine aminotransferase may also serve as a myopathic criterion (*Tollersrud & Ribe* 1967). High levels of these enzymes, however, will not distinguish between a tissue damage of myocardial and skeletal origin, whereas a liver injury in sheep will give high aspartate and normal alanine aminotransferase serum values.

The LDH_5 fraction with high relative mobility found in sheep liver is, when stored as a supernatant, very sensitive to low as well as to elevated temperatures. This isoenzyme appears to be

absent in serum. In horses, *Gerber* (1966) found six LDH fractions in liver tissue. The most cathodic, resembling the LDH_5 in sheep described in the present article, was not released to the blood in a case of liver cell injury. *Fujimoto et al.* (1968) have described an abnormal LDH_5 fraction from human liver tumour tissue migrating significantly faster than its normal counterpart. The liver tissue used in the present experiment, however, originated from apparently healthy animals.

In cattle a great discrepancy exists between the present results and the level of "heat stable" LDH found in serum of calves by *Roussel & Stallcup* (1967). In their experiments on 114 bull calves between 1 and 97 weeks old an average total LDH value of 1834 ± 63 B-B units was obtained during the winter season, which corresponds very closely to our results, 1831 ± 157 B-B units. Heat stable and heat labile LDH was in their experiment determined according to the procedure outlined by *Berger & Broida*. The heat stable fraction after preincubation at 65 °C was found to be 28.9 % and the heat labile fraction lost at 57 °C was 22.8 % of total LDH. Our corresponding results were 2.8 % and 30.5 %. The difference in results cannot be due to addition of $NADH_2$, since the method does not include this component. Nor is the discrepancy due to the fact that the isoenzyme numbering in American and European literature may cause confusion. Most European investigators have followed the example of *Wieland & Pfleiderer* (1957) in assigning number 1 to the isoenzyme with greatest anodic mobility (heat stable) while the majority of the American workers, such as *Berger & Broida*, describe the slowest component (heat labile) as LDH_1. The present results are in agreement with those of *Roussel & Stallcup* indicating that LDH heat stability might be influenced by age. This phenomenon, however, was most pronounced in sheep. In cattle no difference existed in total LDH stability at 65 °C between serum from cows of an average age of 8.2 years and from calves three and a half months old.

Since optimal conditions differ for individual isoenzymes and since serum from different animals contains varying amounts of individual isoenzymes, it is difficult to achieve an LDH assay ideal in all cases.

Determination of serum α-hydroxybutyrate dehydrogenase activity as an alternative to LDH_1, the most heat stable socalled heart fraction, does not seem to be relevant in domestic animals.

Both in cattle and sheep, as earlier shown for pig serum, HBD is far better correlated to total LDH than to the most thermostable LDH isoenzyme.

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SUMMARY

The heat stability of lactate dehydrogenase (LDH) has been investigated in serum from young and adult cattle and sheep. The thermoresistance of the isoenzymes was determined by electrophoresis of serum samples preincubated at different temperatures. Marked differences were found in the percentage distribution of isoenzymes in serum from the two species as well as in the heat stability. LDH in serum from sheep was inactivated at a lower temperature than that in serum from cattle, and inactivation occurred at a lower temperature in young than in adult animals. The enzyme was in both species less tolerant to elevated temperatures than what is reported for human serum. Procedures worked out for a so-called relative heat stability test of LDH in human clinical diagnosis may therefore give misleading results if they were applied uncritically to sera from these animals.

The LDH isoenzyme pattern of some main organs in calves and sheep indicates that a serum heat stability test may be useful in the diagnosis of skeletal muscle injuries in sheep. In cattle the tissue isoenzyme distribution is assumed to be too uniform to give information about specific organ lesions either by serum electrophoresis or by a heating technique.

In contrast to what has been reported in man, serum levels of α-hydroxybutyrate dehydrogenase (HBD) in cattle and sheep, as earlier reported in swine, are found to be far better correlated to total LDH than to the most thermostable isoenzyme, LDH_1.

SAMMENDRAG

Varmestabilitet av laktatdehydrogenase og dens isoenzymer i serum av unge og voksne storfe og sauer. Vurdering av nytten av en relativ varmestabilitetstest og bestemmelse av serum-α-hydroksybutyratdehydrogenase i klinisk diagnostikk.

Varmestabiliteten av laktatdehydrogenase (LDH) er undersøkt i serum fra unge og voksne dyr av storfe og sau. Termoresistensen av de ulike isoenzymer er bestemt ved elektroforese av serumprøver preinkubert ved forskjellig temperatur. Tydelig forskjell ble funnet i den prosentiske fordeling av isoenzymene i serum fra de to arter, og likedan med hensyn til varmestabilitet. LDH i serum fra sau ble inaktivert ved en lavere temperatur enn i serum fra storfe. Hos unge dyr skjedde inaktiveringen før enn hos voksne. Hos begge arter var enzymet mindre resistent ved forhøyede temperaturer enn det som er beskrevet for humant serum. Analysemetoder som er utarbeidet for en såkalt relativ varmestabilitetstest av LDH i humanklinikken, kan derfor neppe gi pålitelige resultater hvis de uten videre blir benyttet på serum fra de nevnte dyrearter.

LDH isoenzymmønsteret i noen av de viktigste organer fra kalver og sauer indikerer at en varmestabilitetstest kan være et brukbart diagnostisk hjelpemiddel ved sykelige forandringer i skjelettmuskulaturen hos sau. Hos storfe er vevsfordelingen av isoenzymer så lik at hverken en serumelektroforese eller en varmestabilitetstest ansees for å kunne gi pålitelige opplysninger om en spesifikk organforandring.

I motsetning til hva som er beskrevet fra humanmedisinen, er serumnivået av α-hydroksybutyratdehydrogenase (HBD) hos kalv og sau, liksom tidligere vist for gris, langt bedre korrelert med total LDH enn med det mest varmestabile isoenzym, LDH_1.

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