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From the Department of Animal Husbandry and Genetics,
Veterinary College of Norway, Oslo.

EFFECTS OF PHYSICAL STRESS ON SERUM ENZYMES AND OTHER BLOOD CONSTITUENTS IN SHEEP

By
S. Tollersrud, B. Baustad and K. Flatlandsmo

The effects of physical exercise on different blood constituents have been more thoroughly investigated in man and laboratory animals than in domestic animals. It has been shown that strenuous physical activity, in untrained individuals particularly, can influence several blood components quite strongly.

The present investigation was carried out to see how far stressing factors connected with transfer of sheep from the lowland to mountain pastures would be reflected in changes of serum enzymes, blood minerals, and other components. It was of interest to ascertain, if differences in reaction could be observed in lambs and ewes taken directly from indoor feeding compared with animals which beforehand had been on pasture.

The sheep were transported by lorry for six hrs. and subsequently herded through mountainous terrain for three hrs.

The following serum enzymes were determined: Aspartate aminotransferase (EC 2.6.1.1), alanine aminotransferase (EC 2.6.1.2), lactate dehydrogenase (EC 1.1.1.27) and its five isoenzymes, α-hydroxybutyrate dehydrogenase, and alkaline phosphatase (EC 3.1.3.1). Further were determined the concentrations of calcium, inorganic phosphorus, magnesium, blood sugar, total serum proteins, and haemoglobin.

MATERIAL AND METHODS

Animals

Of the 37 animals investigated, nine ewes and six lambs had been turned out to pasture in the middle of May. The remaining

10 ewes and 12 lambs were fed indoors until the day of the journey, June 27th. The lambs were at this time eight to 10 weeks old, the mean live weights of the indoor lambs being 22 kg and of outdoor lambs 18 kg. All the ewes, of which some were gimmers, were control animals in experimental studies of intestinal parasites, and were clinically healthy.

The indoor feeding during the last six weeks had consisted only of hay and relatively large amounts of concentrates, the lambs having had access to the concentrate mixture.

Blood sampling, transportation, and herding

All animals were bled in the evening before departure. They were transported 254 km in a double-decked lorry, closely packed, from 9 p.m. to 3 a.m. The last two hrs. of transport took place on a rather bumpy and winding mountain road. After unloading a second blood sample was taken, and the 3-hrs. journey on foot was begun. The animals were herded in a moderate speed, but without rest and with little access to grass. The altitude was about 1,200 m above sea level. During herding some of the animals showed signs of exhaustion, and two lambs had to be carried for long distances. A third blood sample was taken at the end station. Samples from the second and third collections were put in a thermobag with ice, centrifuged in the forenoon, and kept in a refrigerator overnight.

Analytical procedure

The following serum enzymes were determined according to the procedures of the Sigma Chemical Company, St. Louis, USA: Aspartate aminotransferase (AspAT = GOT), and alanine aminotransferase (AlAT = GPT) as described in Sigma Technical Bulletin No. 505 (1964). The activity is expressed as Sigma-Frankel (S-F) units per ml. α-hydroxybutyrate dehydrogenase (HBD) as outlined in Sigma Technical Bulletin No. 495 (1964), expressed as Sigma units of HBD. Total lactate dehydrogenase (LDH) according to Sigma Technical Bulletin No. 500 (1960), expressing the results in Berger-Broida (B-B) units. Isoenzymes of LDH were determined electrophoretically as described by *Baustad & Tollersrud* (1969), the fractions LDH_1 to LDH_5 in order from the anode.

Alkaline phosphatase was determined according to the procedure of *Bessey et al.* (1946), the activity expressed as Bessey-Lowry (B-L) units per ml.

Calcium, magnesium and blood sugar were determined in plasma by autoanalyzer technique: calcium according to the procedure de-

scribed by *Halse* (1967), magnesium by the method of *Gitelman et al.* (1966), and blood sugar according to the modification of *Hoffmann* (1937) described in the Technicon autoanalyzer manual.

Inorganic phosphorus was determined in serum according to a modification (*Zeiss-Opton* 1951) of the method of *Fiske & Subbarow* (1925), total serum proteins by the biuret method, and haemoglobin according to the cyanmethaemoglobin method.

The analytical data were treated statistically by computer technique to test the significance of differences between groups of animals and between times of sampling (Student's t-test).

RESULTS

The main results are given in Tables 1 and 2. The sheep fed indoor until the day of journey are in the following termed "indoor" animals, while those taken from pasture are called "outdoor" animals.

It appears from the tables that increasing values of most serum enzymes could be observed during transportation and the subsequent herding. The elevation, which varied between groups, was much more pronounced in "indoor" lambs than in other animals. Significant increases from the initial levels were thus found in aspartate aminotransferase, α-hydroxybutyrate dehydrogenase, and total lactate dehydrogenase. An average increase of about 50 % was recorded in serum activity of these enzymes at the last sampling. The isoenzymes LDH_3 and LDH_4 were also significantly raised, the LDH_4 fraction in "indoor" ewes as well.

The changes in blood minerals were most distinct after the second bleeding. Highly significant decreases were then observed in phosphorus and magnesium concentrations of the lambs. The values tended to normalize during the walk. Blood sugar showed a continuous, significant increase in "indoor" lambs, while total serum protein was significantly lowered at the end of the journey.

Animals showing symptoms of fatigue and exhaustion during the mountain herding, mainly five ewes and four lambs, all belonged to the "indoor" groups. A comparison of blood values of the laggards with their more unaffected group mates indicated that the greatest changes had occurred in the former animals, particularly in the lambs.

Two lambs and four ewes of the heavily stressed animals showed varying degrees of haemolysis. This was observed in serum from the same individuals at the second and third sampling. Since serum enzyme activities of the respective ewes were

Table 1. Blood values of lambs, initially (I), after transportation (II), and after herding (III). Group means ($\bar{x}$) and standard errors (s).

Blood components	"Indoor" lambs						"Outdoor" lambs					
	I		II		III		I		II		III	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
AspAT, S-F units	93.9	10.2	109.3	10.6	135.2*	13.8	71.8	3.8	77.4	6.0	88.0*	2.4
AlAT, S-F units	17.1	3.0	23.4	3.6	27.0	3.9	10.6	1.3	9.6	1.2	12.6	2.7
HBD, Sigma units	287	29	356	25	430**	35	216	16	302	37	273	29
LDH, B-B units	1115	75	1296	81	1582**	120	926	63	1116	98	1147	94
LDH_1, calc. units	442	29	445	27	480	32	372	19	404	12	382	11
LDH_2, calc. units	141	16	161	18	185	18	113	13	143	20	151	18
LDH_3, calc. units	346	29	453*	40	518**	40	305	46	369	38	393	40
LDH_4, calc. units	140	14	196*	17	283**	36	124	22	184	44	179	41
LDH_5, calc. units	46	14	41	18	116	35	12	4	16	4	42	16
Alk. ph., B-L units	6.02	0.74	6.40	0.89	6.91	0.85	5.48	1.84	5.17	1.36	5.12	1.41
Ca, mg/100 ml	8.93	0.23	9.71	0.29	8.06*	0.31	10.02	0.24	9.90	0.09	9.97	0.40
P, mg/100 ml	9.78	0.33	7.18***	0.30	8.10*	0.50	8.99	1.09	7.64	0.98	8.15	1.11
Mg, mg/100 ml	2.18	0.05	1.70***	0.04	1.99*	0.06	2.29	0.04	1.96***	0.05	2.34	0.05
Protein, g/100 ml	5.48	0.09	5.65	0.10	5.11*	0.13	5.75	0.36	5.55	0.41	5.89	0.46
Glucose, mg/100 ml	61.5	1.7	75.2***	3.2	75.9***	3.1	70.7	5.6	63.1	3.6	71.3	6.9
Hb, g/100 ml	10.8	0.4	10.7	0.4	10.6	0.5	11.1	0.5	12.0	0.6	11.3	0.5

*** indicates significant difference from initial values $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

Table 2. Blood values of adult sheep, initially (I), after transportation (II), and after herding (III). Group means ($\bar{x}$) and standard errors (s).

Blood components	"Indoor" ewes						"Outdoor" ewes					
	I		II		III		I		II		III	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
AspAT, S-F units	87.6	6.5	90.8	5.3	82.2	4.7	102.3	5.9	101.1	4.4	100.7	5.4
AlAT, S-F units	11.3	1.0	16.4*	1.5	13.6	1.1	11.0	0.4	11.5	0.6	11.4	0.6
HBD, Sigma units	261	16	282	17	294	17	248	22	288	26	322	33
LDH, B-B units	1034	53	1033	46	1104	38	1013	61	1049	52	1141	52
LDH_1, calc. units	518	16	518	22	482	15	473	25	486	20	470	25
LDH_2, calc. units	75	6	75	5	90	5	93	6	94	8	112	7
LDH_3, calc. units	298	22	317	16	334	15	314	21	349	21	366	18
LDH_4, calc. units	95	17	107	11	181**	23	105	19	89	11	149	23
LDH_5, calc. units	48	17	16	5	17	3	28	9	31	7	44	10
Alk. ph., B-L units	3.04	0.28	3.24	0.54	3.30	0.26	4.56	0.73	3.86	0.58	4.82	0.85
Ca, mg/100 ml	8.78	0.27	9.13	0.20	9.28	0.20	10.11	0.34	10.09	0.37	9.80	0.34
P, mg/100 ml	6.43	0.42	5.45	0.40	5.82	0.39	4.99	0.35	4.10*	0.18	4.77	0.36
Mg, mg/100 ml	2.45	0.05	2.43	0.11	2.40	0.09	2.53	0.06	2.19	0.17	2.55	0.09
Protein, g/100 ml	6.23	0.10	6.56	0.13	5.99	0.15	6.67	0.13	6.97	0.12	6.43	0.15
Glucose, mg/100 ml	49.0	1.8	52.6	2.4	46.5	2.7	55.7	2.3	59.8	3.1	48.2	3.0
Hb, g/100 ml	11.4	0.2	11.3	0.3	11.4	0.3	11.0	0.4	10.4	0.8	10.8	0.5

*** indicates significant difference from initial values $P < 0.001$, ** $P < 0.01$ * $P < 0.05$.

changed very little, it is assumed that the elevated values of the lambs were due to factors other than the haemolysis.

Of interest, but not directly shown in the tables, are the following significant differences between groups at the initial stage:

Aspartate aminotransferase was higher in ewes than in lambs. This was found in animals on pasture, but not on indoor feeding. The observation may support previous findings of an increased serum transferase activity in grazing animals (*Tollersrud & Gedde-Dahl* 1971). The percentage fraction of LDH_1 was lower, and of LDH_2 and LDH_4 higher in lambs than in ewes, indicating that the adult LDH isoenzyme pattern was not yet attained in lambs at this age.

Alkaline phosphatase, inorganic phosphorus, and blood sugar levels were higher, while magnesium and total serum proteins were lower in lambs than in adult ewes. Animals fed indoors showed lower calcium and higher phosphorus concentrations than those on pasture.

DISCUSSION

It is generally believed that an elevated enzyme activity in blood serum after physical excercise is due to an increased cellular membrane permeability. The permeability has been shown to increase under the influence of factors such as hypoxia, insulin, and adrenal hormones (*Hess* 1963). Physical exercise at simulated high altitudes has caused greater serum enzyme elevations than at sea level (*Altland et al.* 1968). How far and in what way endocrine systems are involved in the enzyme release from cells to blood is not fully known. The following mechanism may be possible: Stress → endocrine reaction → increased cellular permeability → enzyme release.

Cell damage in the usual sense as a cause of enzyme leakage seems to be less probable under these circumstances, since recovery to normal levels usually occurs within a few hrs. after cessation of the physical load (*Halonen & Konttinen* 1962).

In the present observations it was clearly demonstrated that the greatest serum enzyme increase appeared in lambs which were unaccustomed to muscular activity. The obvious shift of LDH isoenzyme distribution in serum towards a pattern more rich in intermediate and cathodic fractions points to the assumption that the main origin of enzyme release would be ske-

letal muscle. As earlier shown (*Tollersrud* 1970) skeletal muscle tissue of sheep is more rich in cathodic LDH isoenzymes than any of the other main organs. A significant increase of the LDH_5 fraction, however, was not obtained, partly due to great individual variations within the group. LDH_5 is considered to be the most labile of the LDH isoenzymes, in vivo as well as in vitro.

Hyldgaard-Jensen (1967) reports a clear tendency towards increased appearance of the cathodic LDH isoenzymes in plasma obtained from pigs just before killing at the slaughter-house compared with samples taken at the farm.

Observations by *Manz* (1964) of slaughter-pigs showed an increased total LDH activity with increasing distances of transportation. *Prange et al.* (1966) found, in cattle, serum elevations of LDH attributable to transport. Young animals appeared more sensitive in this respect than older ones.

Initial levels of serum enzymes and LDH isoenzymes of adult sheep are in agreement with data reported earlier (*Tollersrud* 1969, *Tollersrud* 1970). The present values of total LDH and LDH isoenzymes in the lambs differ, however, from the results previously obtained in newborn and in 10-day-old lambs (*Tollersrud & Baustad* 1970). LDH and its isoenzyme fractions in serum of sheep obviously depend greatly upon the age of the animals, as shown in the following summary of previous and present results in Table 3.

Table 3. Total LDH and LDH isoenzymes of sheep at different ages.

Age	Total LDH	Percentage and calculated values of LDH isoenzymes				
		LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
10 hrs.	1530	23.1 (353)	12.9 (197)	41.1 (628)	23.0 (352)	0 (0)
10 days	1452	29.5 (428)	16.6 (241)	35.8 (520)	18.1 (263)	0 (0)
10 weeks (indoor)	1115	39.6 (442)	12.7 (141)	31.0 (346)	12.6 (140)	4.1 (46)
Adults (indoor)	1034	50.1 (518)	7.3 (75)	28.8 (298)	9.2 (95)	4.6 (48)

Total LDH and the intermediate isoenzyme fractions of physically stressed lambs showed a trend towards values previously observed in younger lambs.

The causal factor of haemolysis in some of the heavily stressed animals is not known. The considerable fall occurring in magnesium and inorganic phosphorus concentrations during the transport, may be contributing factors to the collapse in

some of the "indoor" animals, and to cases of tetany seen in previous years. The most critical period is empirically shortly after the start of herding, coinciding with the lowest observed blood mineral levels. Calcium injections have proved to be of little effect. This may be in accordance with the present results, showing that blood calcium levels were not decreased after the transportation.

The investigation supports previous practical experiences saying that well-fed animals taken directly from indoors are more susceptible to physical break-down during transfer to the mountains than those previously acclimatized on pasture.

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SUMMARY

In connection with transfer of sheep from the lowland near Oslo to mountain pastures at an altitude of 1,200 m above sea level, investigations were carried out in 37 animals to study the effect of physical stress on serum enzymes and other blood constituents. The sheep were adult ewes and lambs. About half of the animals had been accustomed to outdoor life on pasture for more than one month, while the others were moved directly from indoor feeding. Blood was collected before departure, after six hrs. of long-distance transportation by lorry, and after three hrs. of subsequent continuous herding on foot. The following blood components were determined: Aspartate aminotransferase (AspAT = GOT), alanine aminotransferase (AlAT = GPT), α-hydroxybutyrate dehydrogenase (HBD), total lactate dehydrogenase (LDH), LDH isoenzymes, alkaline phosphatase, calcium, inorganic phosphorus, magnesium, blood sugar, total serum proteins, and haemoglobin.

In summary, it may be said that the lambs reacted with greater changes of the blood components than adult animals, and that untrained, indoor fed lambs were distinctly more sensitive than those

taken from pasture. The "indoor" lambs showed a statistical significant increase from the initial values in AspAT, HBD, total LDH, the isoenzymes LDH_3 and LDH_4, and blood sugar. Significantly decreased values were recorded in Ca, P, Mg, and total serum protein. Some of these changes, as in Mg and P, were most pronounced after transportation, while elevations of serum enzyme levels continued to increase during the subsequent herding.

Based upon the shift in LDH isoenzyme distribution towards a more cathodically dominated pattern it is supposed that the main origin of increased serum enzyme activity was skeletal muscle.

SAMMENDRAG

Virkningen av fysisk påkjenning på serumenzymer og andre blodbestanddeler hos sau.

I forbindelse med fjellsending av sau fra Oslo-området til Iungsdalen i Hallingdal, 1200 m.o.h., er det utført blodanalyser på 37 dyr for å undersøke virkningen av fysisk stress på serumenzymer og andre blodkomponenter. Ca. halvparten av dyrene, både voksne og lam, hadde forut for fjellsendingen gått ute på beite i vel en måned, mens de øvrige ble sendt direkte fra innefôring. Blodprøver ble tatt før sending, etter seks timers lastebiltransport og etter en påfølgende tre timers sammenhengende driving.

Følgende blodbestanddeler ble bestemt: Aspartataminotransferase (AspAT = GOT), alaninaminotransferase (AlAT = GPT), α-hydroksybutyratdehydrogenase (HBD), total laktatdehydrogenase (LDH), LDH-isoenzymer, alkalisk fosfatase, kalsium, uorganisk fosfor, magnesium, blodsukker, total serumprotein og hemoglobin.

Summarisk kan en si at lammene reagerte med større forandringer i blodkomponentene enn voksne dyr, og at lam som var utrenete, reagerte sterkere enn de som var vant til uteliv på forhånd. Utrenete lam viste en statistisk signifikant økning fra utgangsverdiene i AspAT, HBD, total LDH, isoenzymene LDH_3 og LDH_4 samt blodsukker. En signifikant nedgang ble funnet i Ca, P, Mg og totalprotein. Noen av disse forandringene, f. eks. Mg og P, var størst etter transporten, mens serumenzymnivået fortsatte å stige under den påfølgende driving.

Ut i fra den observerte forskyvning i fordelingen av LDH isoenzymer i serum mot et mer katodisk preget mønster, antar en at opprinnelsen til de forøkete serumenzymverdier hovedsakelig var skjelettmuskulatur.

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