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RELATION BETWEEN THE PLASMA LEVEL OF GLUTAMIC-OXALOACETIC TRANSAMINASE AND THE MORPHOLOGICAL STATUS OF SKELETAL MUSCLE IN PIGS¹⁾

By

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Muscular dystrophy (MD) may give rise to a very variable disease pattern (6). The locomotive disturbances of the animals are more or less pronounced or are completely lacking. Many other diseases in pigs may manifest themselves by similar symptoms. For this reason it is of great value to have an objective method for diagnosing MD. It is also essential to have a good criterion of the development of the disease in treatment experiments. Earlier, a good agreement was found between the plasma level of glutamic-oxaloacetic transaminase and the findings at histological examination of biopsy specimens or at autopsy (7), but variations of this relation have also been reported (3).

In the experiment reported here one more comparative investigation has been made of the plasma-GOT level in pigs given a MD-provoking diet and the morphological condition in their muscles. The latter examination was made partly as biopsies, partly as complete postmortem examinations.

MATERIAL AND METHODS

The animals used in the experiment were pigs of the Swedish Land Breed, females as well as castrated males, which weighed

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20—25 kg. They were bought from farms known to be free from contagious diseases. Each pig was given 100,000 I.U. of vitamin A and 250,000 I.U. of vitamin D₂ as an intramuscular injection at the beginning of the experiment. Litter mates were as far as possible distributed equally among the groups, since variations are observed in the disposition for MD in different litters (4).

The diet consisted of grains to which 3 % cottonseed oil was added. The grain, consisting of oats and barley in equal parts, was heated at 100°C for 24 hours under continuous flow of air, which lowered its vitamin-E level from initially 30—50 mg. to about 5 mg. per kg dry weight (8). In a similar manner the amount of tocopherol of the oil was reduced from 800—900 mg. to 30—40 mg. per kg. (4). Just before feeding, the grain and the oil were thoroughly mixed in a food mixer. The animals had free access to water.

Determinations of glutamic-oxaloacetic transaminase (GOT) by the method of Reitmann and Frankel and ornithine-carbamyl transferase (OCT) by Reichard's method were made in blood plasma as described in a previous paper (9). Normal limits of GOT are 0—59 units and of OCT 1—11 units in pigs. The plasma level of GOT was determined at brief intervals and OCT was examined every time GOT was elevated.

Biopsy specimens of the skeletal muscles of the pigs were taken from the longissimus dorsi under trichloroethylene narcosis. Two specimens, measuring 2×1×0.5 cm., were taken at the same time. One was immediately fixed in absolute ethyl alcohol and the other one an hour later in 10 % formalin solution.

Complete postmortem examination was made in 25 of the 36 pigs. Four of them had died spontaneously. The others were killed by intravenous injection of 6 % solution of pentobarbitone sodium and the autopsies were performed on bodies that were still warm. Material for histological examination was taken from skeletal muscles (the longissimus dorsi, the semimembranosus, the rectus abdominis, the triceps brachii [caput longum], and diaphragm), heart muscle (right and left ventricle), liver and kidney. This material was fixed in the same manner as the biopsy specimens.

The material for histological examination was cut in a frozen state as well as embedded in paraffin and sectioned, and was stained with Scharlach Rot and with haemalum-eosin. In order

to demonstrate glycogen the material, which was fixed in absolute ethyl alcohol, was stained with Best's carmine stain.

The division into groups was made on day 12, when half the pigs had reacted with GOT elevations. The distribution was done so that each group consisted of an equal number of reacting and nonreacting animals. The pigs were treated as follows. Group I: 0.06 mg. of selenium (part of which, corresponding to 200 μ C, was given as Se⁷⁵-tagged salt) on day 14. Group II: 3 mg. of α -tocopheryl acetate + 0.06 mg. of selenium on day 13. Group III: 6 mg. of α -tocopheryl acetate + 0.06 mg. of selenium on day 13. Group IV: No treatment. All the doses refer to 1 kg. of body-weight. The treatment was given as an intramuscular injection. (The results concerning the elimination rate of selenium in pigs will be published separately, as will the results of the treatment [2, 8]).

The preparations used for therapy were vitamin E as a water suspension of α -tocopheryl acetate (16.67 mg. per ml.), and selenium as a water solution of sodium selenite, Na₂SeO₃ · 5 H₂O (0.66 mg. per ml. corresponding to 0.2 mg. of Se).

RESULTS

The results are summarized in tables 1—3.

In no case did macroscopical examination of the skeletal muscles show any certain, observable changes. While on the whole, no gross changes were seen at autopsy of the killed pigs, circulatory failure with transudations to the body cavities dominated in those pigs that had died spontaneously. In some pigs there was a slight to moderate parasite invasion by *Ascaris suum* and *Metastrongylus elongatus*, and in a few cases the presence of parasites may have had a debilitating effect on the individual.

At the histological examination of the skeletal muscles waxy muscular degeneration in different stages was observed, and not seldom were these stages seen in the same slice. The acute form was characterized by a swelling and homogenization of the sarcoplasm into a hyaline mass, in which an irregular or discoidal fragmentation could as a rule be seen (Figs. 1 and 2).

In some cases fatty degeneration and dystrophic calcification belonged to the picture. In the subacute state a diminishing of the volume of the muscle fibre and an infiltration with phagocytes and fibroblasts were seen. In this stage the hyaline sub-

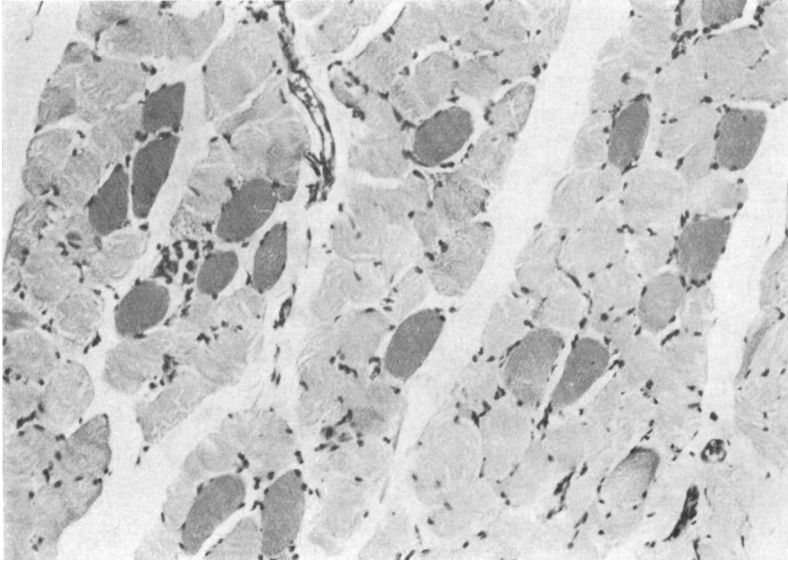


Fig. 1. Waxy muscular degeneration in the form of hyalinization of some cross-cut muscle fibres. The diaphragm. Fig. no. 7. Haemalum-eosin.

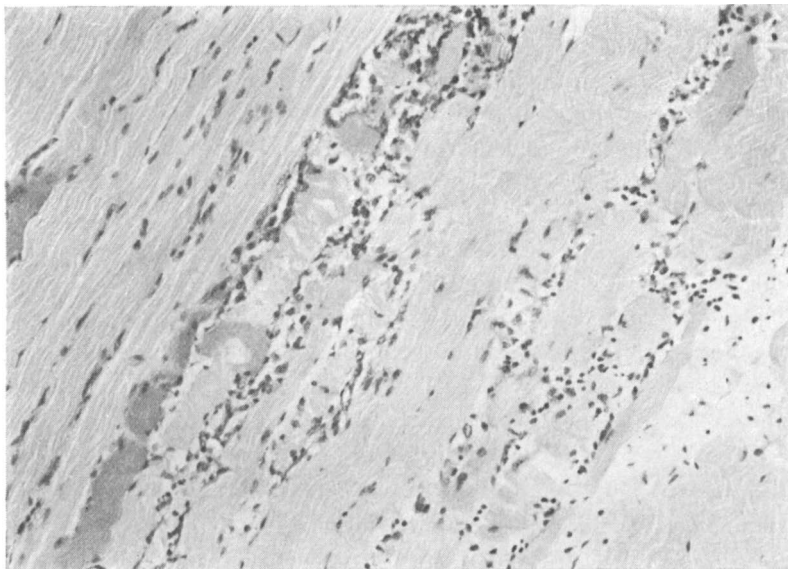


Fig. 2. Waxy muscular degeneration with fragmentation and resorption of hyalinized muscle fibres. The semimembranosus. Fig. no. 7. Haemalum-eosin.

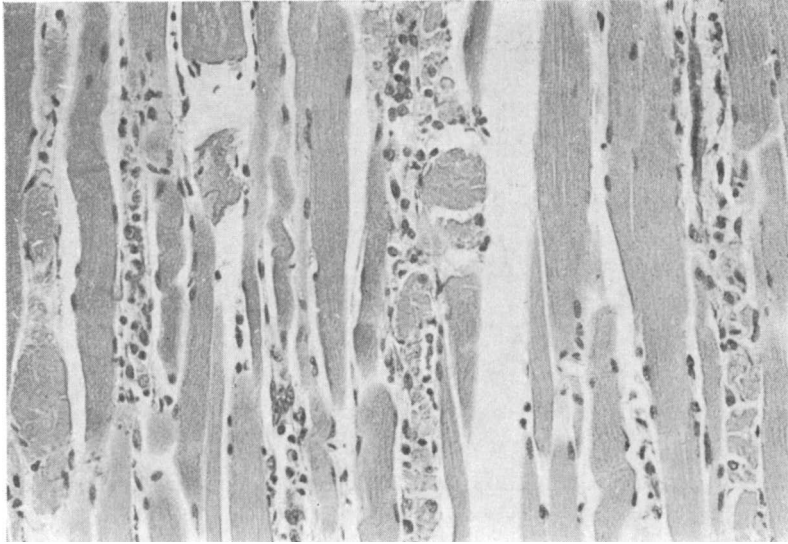


Fig. 3. Waxy muscular degeneration with fragmentation and resorption of hyalinized muscle fibres together with proliferation of sarcolemma nuclei. The rectus abdominis. Pig no. 24. Haemalum-eosin.

stance was still observed (Fig. 3). When this was resorbed a healing stage was seen, which was characterized by a proliferation of sarcolemma nuclei and a development into a regeneration of the muscle fibre.

In solitary pigs the liver showed changes of the kind that occurs in liver dystrophy (hepatosis diaetetica) with centrolobular liver necroses of different size (5).

The amount of glycogen in the different organs showed, as a rule only small variations. The centrolobular necroses in the liver, however, contained very scanty glycogen, contrary to the intact liver tissue. In the skeletal muscles a reduced glycogen level could be established with certainty in one pig only (no. 30), whose musculature showed pronounced waxy degeneration.

DISCUSSION

As seen in Table 1 there is not complete conformity between the plasma-GOT level and the status of skeletal muscles at the time of taking biopsy specimens. However, an absolute agreement could not be expected. Pathological changes may be present in

such a small part of the musculatur, that they will not give rise to the release of sufficient GOT to cause an elevated plasma level of the enzyme. The biopsy specimens constitute only a very small part of the skeletal muscles, whereas the amount of GOT in plasma reflects the state in the whole musculature.

Biopsy specimens were taken from the longissimus dorsi, because, from a technical viewpoint, this muscle is most suitable for operation. Histological examination of biopsy specimens taken at the same time from the longissimus dorsi, the semi-membranosus and the triceps brachii has earlier proved to give corresponding results (7). Likewise, in the material examined here the tendency was the same. The numbers of autopsies with MD in these three muscles were 11, 13 and 12 respectively. A higher frequency of MD was, however, observed in the diaphragm and the rectus abdominis, with 16 and 15 cases respectively. These muscles, which in relation to other muscles have a high activity, show a high frequency of affection in other kinds of muscular dystrophy as well (1). To a certain degree, the above-mentioned lack of agreement between the transaminase level and histological findings in biopsy specimens might be associated with the varying frequency of MD in different muscles.

In contrast to the examination of biopsy specimens, a comparison between GOT values and the presence of MD at autopsy shows a good conformity (Table 2). However, there was one exception (pig no. 36). The non-conformity in this case may depend on the fact that this pig was killed so soon after the beginning of the experiment that a slight MD was not reflected in elevated GOT values. Judging from the GOT elevations, 3 pigs had been affected with MD. However, GOT had returned to a normal level after treatment, whereas a histologically demonstrable MD was present at the autopsy performed 2 to 15 days later. Experiments with infusion of GOT have demonstrated that the circulating enzyme disappears after 1 to 5 days (9). The results in the autopsy material strongly indicate that MD is present when GOT is elevated and OCT normal.

The material of liver dystrophy is too small to allow any conclusions. Of the 3 cases in which the diagnosis was verified at autopsy, 2 had raised levels of both GOT and OCT.

REFERENCES

1. *Adams, R. D., Denny-Brown, D. and Pearson, C. N.*: Diseases of muscle. Hoeber, New York, 1953.
2. *Ekman, L., Orstadius, K. and Åberg, B.*: Distribution of Se^{75} -tagged sodium selenite in pigs with nutritional muscular dystrophy. *Acta vet. scand.* 1963, 4, 92—96.
3. *Grant, C. A.*: Morphological and aetiological studies of dietetic microangiopathy in pigs. *Acta vet. scand. Suppl.* 3. Stockholm, 1961.
4. *Lindberg, P. and Orstadius, K.*: Production of muscular dystrophy in pigs by feeding cottonseed oil. *Acta vet. scand.* 1961, 2, 226—235.
5. *Obel, A. L.*: Studies on the morphology and etiology of so-called toxic liver dystrophy (Hepatositis diaetetica) in swine. *Acta path. microbiol. scand. Suppl.* 94, Lund, 1953.
6. *Orstadius, K.*: Nutritional muscular dystrophy in pigs. Studies on the aetiology, diagnosis and therapy. Thesis. Stockholm, 1961.
7. *Orstadius, K., Wretlind, B., Lindberg, P., Nordström, G. and Lannek, N.*: Plasma-transaminase and transferase activities in pigs affected with muscular and liver dystrophy. *Zbl. Vet. Med.* 1959, 6, 971—980.
8. *Orstadius, K., Nordström, G. and Lannek, N.*: Combined therapy with vitamin E and selenite in experimental nutritional muscular dystrophy of pigs. In press.
9. *Wretlind, B., Orstadius, K. and Lindberg, P.*: Transaminase and transferase activities in blood plasma and in tissues of normal pigs. *Zbl. Vet. Med.* 1959, 6, 963—970.

SUMMARY

A comparative investigation of the amount of plasma glutamic-oxaloacetic transaminase (GOT) and the morphology of the skeletal muscles has been made in pigs which were fed a muscular-dystrophy-provoking diet. A qualitatively good correlation was seen on comparing the enzyme level and autopsy findings. However, slight muscular changes may probably appear without being reflected in elevated transaminase values. A less good agreement was observed between GOT values and histological findings in biopsy specimens from the longissimus dorsi. Yet, from the autopsy material it was found that this muscle was not the most adequate measure of MD in pigs.

8	31	29	27	33	47	47	43	29	23	33	33	29	
			+									+	
26	31	36	44	140	536	202	44	16	22	31	35	54	
5	28	27	23	57	250	290	62	18	31	43	27	35	83
					+								
21	29	28	16	41	46	60	29	20	30	44	58	76	193
									+				
25	35	22	17	28	78	104	40	20	19	28	23	52	110
					+								
4	28	39	17	38	68	113	32	28	18	19	19	23	36
									+				
III	1	25	27	19	25	20	16	15	14	18	16	20	20
9	38	28	44	33	33	44	28	27	19	33	35	31	57
									+				
19	25	18	16	41	122	78	43	15	14	18	19	35	78
28	54	57	173	900	536	380	80	39	19	49	49	39	31
			+						+				
IV	36	36	23	33									
			+										
29	36	35	72	210									
3	25	20	19	41	76	135							
					+	+							
31	38	51	93	788	616	380							
24	23	20	18	55	154	428	380	867					
								+					
27	39	49	36	46	97	180	208	282	452				
									+				
7	39	46	25	51	59	71	100	140	214	208	266	220	
									+			+	
11	23	30	31	25	23	16	185	14	31	61	163	70	29
									+				
15	23	25	19	31	28	28	23	36	65	61	140	258	520
									+				
2	31	28	19	60	135	156	242	250	540	616	266	116	62
									+				

Table 2. Histological examination of muscles taken at biopsies and The histological changes of the skeletal muscles are graduated quantitatively. degeneration. 1) indicates that the animal had died spontaneously. The other pigs barbitone-sodium solution.

Group no.	Pig no.	BIOPSY				POST-MORTEM EXAMINATION						
		Day	Waxy muscular degeneration			Day	Waxy muscular degeneration					
							The long. dorsi			The semimembr.		
			acute	sub-acute	healing		acute	sub-acute	healing	acute	sub-acute	healing
I	6	7	0	0	0	15	0	0	0	0	0	0
	34 ¹⁾	7	++	0	0	15	0	0	0	+	0	0
	10	7	0	0	0	17	+	+	0	+	++	+
		14	0	0	0							
	30	7	++	0	0	17	0	0	+	+	++	+++
	12	7	+	0	0	22	0	0	0	0	0	0
	23	1	0	0	0	22	0	0	0	0	0	+
	14	7	0	0	0	29	0	0	0	0	0	0
	22	7	+	0	0	29	+	+	0	+	++	0
	17	1	0	0	0	36	+	+	+	+	++	++
20	7	0	0	0	36	+	+	+	+	+	0	
II	18	7	0	0	0	14	0	0	0	0	0	0
	35	1	0	0	0	14	0	0	0	+	0	0
	16	7	0	0	0	16	0	0	0	0	0	0
		14	0	0	0							
	33	1	0	0	0	16	0	0	0	0	0	0
		14	0	0	0							
	13	1	0	0	0	21	0	0	0	0	0	0
		14	0	0	0							
	32	7	++	0	0	21	0	0	0	0	0	0
		14	+	0	0							
	8	7	+	0	0	28	0	0	0	0	0	0
		14	0	0	0							
	26	7	0	0	0	28	0	0	0	0	0	0
		14	0	0	0							
		21	0	0	0							
	5	1	0	0	0	28	0	0	0	0	0	0
		14	+	0	0							
		21	0	0	0							
		28	0	0	0							
	21	1	0	0	0	28	0	0	0	0	0	0
14		0	0	0								
21		+	0	0								
28		+	0	0								
25	1	0	0	0	28	0	0	0	0	0	0	
	14	0	+	0								
	21	0	0	0								
	28	0	0	0								

POST-MORTEM EXAMINATION									Other findings
Waxy muscular degeneration									
The rectus abdom.			The triceps brachii			The diaphragm			
acute	sub-acute	healing	acute	sub-acute	healing	acute	sub-acute	healing	

+	0	0	+	0	0	+	+	0	Dietetic hepatitis Parasitic bronchitis
++	++	0	+	0	0	++	+	0	
+	+	+	+	0	0	+	+	++	Ascariasis Parasitic bronchitis Ascariasis
+	+	0	0	+	+	++	+	0	
+++	++	++	++	++	+	++	+	0	Parasitic bronchitis
+	0	0	++	+	++	+	+	++	
+	0	0	++	+	+	++	+	+	

Table 3. Plasma OCT level and presence of liver dystrophy (hepatosis diaetetica) at post-mortem examination. Only those pigs that deviated from the normal status are included in this table.

Normal limits of OCT are 1—11 units. The presence of hepatosis diaetetica is marked with +, while — indicates no liver dystrophy. ¹⁾ indicates that the animal had died spontaneously. The other pigs that were examined post-mortem were killed by intravenous injection of pentobarbitone-sodium.

For division into groups and treatment, see the text.

Group no.	Pig no.	Day	Plasma-OCT level	Post-mortem examination	
				Day	Hepatitis diaetetica
I	6			15	+
	22	14	13,5		
		16	11,5	29	—
	34 ¹⁾	7	40,0		
		10	41,0		
		12	46,5		
		14	70,5		
	15	107,0	15	+	
IV	2	21	18,6		
		23	15,4		
		26	14,3	—	
	11	28	11,0	—	
	24 ¹⁾	17	18,5	17	—
	29 ¹⁾	10	34,4	12	+

autopsies. The biopsy specimens are taken from the longissimus dorsi.
 +++ indicates extensive, ++ moderate, + slight, and 0 no waxy muscular
 that were examined post mortem were killed by intravenous injection of pento-
 For division into groups and treatment, see the text.

POST-MORTEM EXAMINATION									Other findings
Waxy muscular degeneration									
The rectus abdom.			The triceps brachii			The diaphragm			
acute	sub-acute	healing	acute	sub-acute	healing	acute	sub-acute	healing	
+	+	0	0	0	0	+	+	+	Diatetic hepatitis
+++	+	0	0	+	++	0	+	++	Diatetic hepatitis Parasitic bronchitis
+	0	0	0	0	0	+	0	0	Focal chronic interstitial nephritis
+	+	++	0	++	++	+	+	+	
0	0	0	0	0	0	0	0	0	
+	+	+	0	+	++	+	+	+	
0	0	0	0	0	0	0	0	0	
+	0	0	0	0	0	0	0	0	
0	0	0	0	0	0	0	0	0	
0	0	0	0	0	0	0	0	0	
+	0	0	0	0	0	+	0	++	Ascariasis
0	0	0	0	0	0	0	0	0	Focal chronic interstitial nephritis
0	0	0	+	0	0	+	+	0	Ascariasis; Parasitic bronchitis
0	0	0	0	0	0	0	0	0	
0	0	0	0	0	0	+	0	0	Ascariasis; Parasitic bronchitis
0	0	0	0	0	0	0	0	0	Ascariasis
+	0	0	0	0	+	+	0	+	Chronic purulent bronchopneumonia

ZUSAMMENFASSUNG

Korrelation zwischen dem Plasma-Gehalt an Glutamin-Oxalessigsäure-Transaminase und der Morphologie der Skelettmuskulatur der Ferkeln.

Vergleichende Untersuchungen zwischen dem Plasma-Gehalt an Glutamin-Oxalessigsäure-Transaminase (GOT) und der Morphologie der Skelettmuskulatur, wurden an Ferkeln die bei einer Muskeldystrophie erzeugender Diät gehalten wurden, durchgeführt. Beim Vergleich zwischen den Befunde vom Enzym-Gehalte und den Obduktionen, wurde eine qualitativ gute Korrelation festgestellt. Wahrscheinlich können auch mildere Muskelschädigungen entstehen ohne dass diese in erhöhten Transaminase-Werten zum Ausdruck kommen. Eine weniger gute Übereinstimmung mit bezug auf die GOT-Werten und die histologischen Befunde bei den Biopsien vom *M. longissimus dorsi* wurde beobachtet. Aus dem Obduktionsmaterial hat sich inzwischen erwiesen, dass dieser Muskel nicht die besten Werte für die Feststellung der Muskeldystrophie der Schweinen liefert.

SAMMANFATTNING

Korrelation mellan blodplasmas halt av glutaminoxalättiksyretransaminas (GOT) och skelettmuskulaturens morfologiska status hos grisar.

En jämförande undersökning av plasmas innehåll av glutaminoxalättiksyretransaminas (GOT) och skelettmuskulaturens morfologi har utförts på grisar, som utfordrats med en muskeldystrofi framkallande diet. En kvalitativt god korrelation sågs vid jämförelse mellan enzymhalt och obduktionsfynd. Sannolikt kan dock lindriga muskelförändringar uppträda utan att detta återspeglas i förhöjda transaminasvärden. En mindre god överensstämmelse iakttoogs avseende GOT-värden och histologiska fynd i biopsier från *M. longissimus dorsi*. Det framgick emellertid av obduktionsmaterialet att denna muskel inte är den bästa värdemätaren på MD hos grisar.

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