Acta vet. scand. 1975, *16*, 368—378.

From the Department of Physiology, Endocrinology and Blood-grouping, Royal Veterinary and Agricultural University, Copenhagen, Denmark.

THE EFFECT OF PHYSICAL TRAINING ON SKELETAL MUSCLE ENZYME COMPOSITION IN PIGS

By

P. Fogd Jørgensen and *J. F. Hyldgaard-Jensen*

JØRGENSEN, P. FOGD and J. F. HYLDGAARD-JENSEN: *The effect of physical training on skeletal muscle enzyme composition in pigs*. Acta vet. scand. 1975, *16*, 368—378. — Running exercise in pigs results in an elevation of lactic acid in blood. This elevation in blood lactate does not occur in physically conditioned pigs. Activities of succinic dehydrogenase, fructose-1,6-diphosphate aldolase, lactate dehydrogenase and creatine phosphokinase as well as the myoglobin content were determined in m. gastrocnemius from 6 ergometer-trained and 4 untrained pigs. The succinic dehydrogenase and myoglobin contents were significantly higher ($P < 0.01$) in trained animals, whereas no changes were noted in the aldolase and creatine phosphokinase contents. The lactate dehydrogenase showed somewhat reduced levels in the trained pigs. This was accompanied by an increased H/M subunit ratio. The results provide evidence for an increase in the maximal aerobic metabolism in trained pigs and that trained pigs to a higher extent can rely on an aerobic energy metabolism during running exercise.

pigs; exercise; training; blood lactic acid; muscle enzymes.

During physical exercise lactic acid is produced in skeletal muscles as a result of the anaerobic breakdown of glycogen and glucose. The formed lactic acid is either oxidized further on to carbon dioxide and water via the Krebs cycle within the muscle or transported into the blood provided the oxidative capacity of the muscle tissue is low.

In pigs running-exercise results in elevated blood lactic acid levels indicating that the oxidative capacity of the muscle tissue for lactic acid is low (*Ludvigsen* 1957, *Sybesma & Hessel-de Heer* 1967, *Van den Hende et al.* 1970, *Bickhardt et al.* 1972). The elevation of blood lactate in response to exercise is less pronounced in normal pigs than in those predisposed for exertional myopathy (*Bickhardt et al.*). This syndrome, which may appear during exertion, is characterized by an abnormally rapidly progressing anaerobic glycolysis in skeletal muscles bringing about increased levels of lactic acid and H^+, while the energy-rich phosphates are exhausted. The syndrome thus corresponds to the changes observed in muscles during the development of the PSE changes ("pale, soft, exudative" changes) post mortem.

Physical conditioning is reported partly to reduce the degree of blood lactate elevation during physical exercise and to prevent the appearance of the aforementioned biochemical changes in skeletal muscles (*Rülcker* 1968, *Lannek et al.* 1973, *Lindberg et al.* 1973a, b).

On the basis of the observed training effects the hypothesis has been put forward that trained pigs exhibit an increased aerobic metabolism in the skeletal muscles. The present investigation comprising determinations of representative enzymes of the aerobic and the anaerobic metabolism in skeletal muscles was carried out aiming to further clarify this hypothesis.

ANIMALS AND METHODS

The investigation comprised 10 pigs, 7 castrated males and 3 gilts originating from 3 different litters and being all of the Danish Landrace breed. Four pigs (1 gilt and 3 males) were used as untrained controls, while the remaining 6 pigs were trained 1 to 3 times daily during periods of 42—83 days. The distances covered in these training programmes ranged from 73 to 319 km.

Training was performed by running on a treadmill (ergometer) driven by a geared electromotor. The run was initiated by a period of 1.5—2.0 min. at a speed of 75 m/min., thereafter the speed increased to 105 m/min. during the rest of the running period, which for 4 pigs lasted 16 min. and for 2 pigs 21 min. After the run the pigs were fed a standard feed mixture (*Palludan* 1966). At the end of the training period a trained and an untrained pig (litter mates) were exposed to a running exercise lasting 17 min. at a speed of 100 m/min. The exercise was carried out 3 times at intervals of 1 day. Blood samples for the determination of lactic acid (*Barker & Summerson* 1941) were collected from an ear vein before and after exercise. Another pair of a trained and an untrained pig (litter mates) was exercised at the end of the training period for 21.5 min. at a speed of 102 m/min. Blood samples were in this case drawn before, during and after exercise from a catheter previously inserted in the vena cava cranialis (*Mount & Ingram* 1971, *Jørgensen* 1974).

Following 24—28 hrs. rest and 18 hrs. deprivation of food the pigs were killed. Two pigs were shot and subsequently bled, 8 pigs were bled during a "Leopental" narcosis. The enzyme composition of the skeletal muscle was not influenced by methods of killing. The pigs ranged in weight from 48 to 85 kg at the time of killing.

Samples from the central part of the caput laterale of m. gastrocnemius were removed immediately after death, then frozen in liquid nitrogen and stored at —20°C.

Quantitative estimations of creatine phosphokinase (CPK), fructose-1,6-diphosphate aldolase (Ald), succinic dehydrogenase (SDH) and myoglobin (Mb) were done spectrophotometrically as described previously (*Jørgensen*). The spectrophotometric determination of lactate dehydrogenase (LDH) as well as the agarosegel electrophoretic estimation of the H- and M-subunits of LDH were carried out according to methods described by *Hyldgaard-Jensen* (1971). The enzyme activity was determined at 25°C and expressed in international units per g wet tissue (u/g).

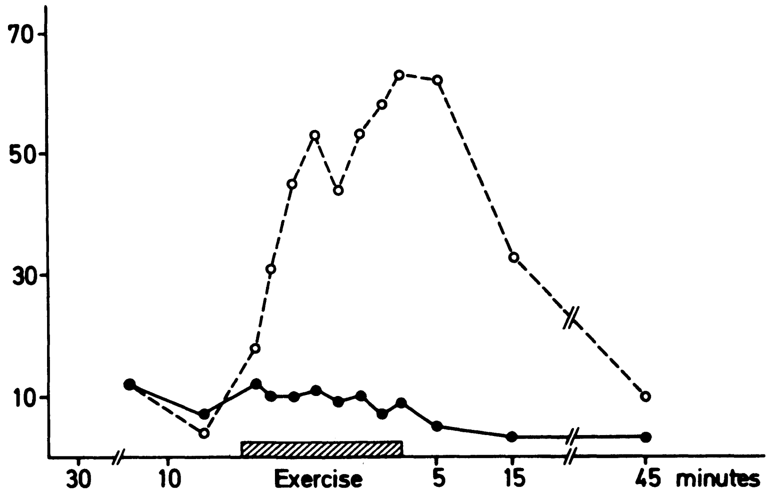
RESULTS

The lactic acid levels in blood before and after exercise (17 min. at a speed of 100 m/min.) in a trained and an untrained pig appear from Table 1. In the untrained pig a marked increase

Table 1. Lactate concentration in blood (mg/100 ml) before and after exercise of 1 trained and 1 untrained pig. (Mean value of 3 exercises ± s.e.m.).

Time of sampling	Trained	Untrained
1 hr. before	19±9	11±3
30 min. before	26±17	8±0
5 min. after	10±1	100±20[a]
30 min. after	10±6	13±1

[a] Trained different from untrained, $P < 0.02$ (Students t-test).



F i g u r e 1. Lactate concentration (mg/100 ml) in blood during and after exercise of a trained (———), and an untrained (- - - - -) pig. (Exercise: 21.5 min., 102 m/min.).

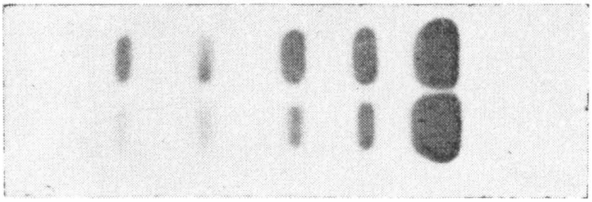
in the lactic acid level is noted 5 min. after exercise, whereas in the trained pig lower levels than pre-exercise levels were observed at the same time. The difference in the lactic acid content between the 2 pigs after exercise is significant ($P < 0.02$).

A similar trend was found after exercise in another pair of trained and untrained litter mates (Fig. 1). During exercise of the untrained pig a biphasic increase in the blood lactic acid level was noted. The first peak appeared 5 min. after start of

T a b l e 2. Creatine phosphokinase (CPK), aldolase (Ald), lactate dehydrogenase (LDH), H-, M-subunit ratio of LDH, succinic dehydrogenase (SDH), and myoglobin (Mb) in m. gastrocnemius. Mean value ± s.e.m. Number of pigs within brackets.

Group of animals	CPK (u/g)	Ald (u/g)	LDH (u/g)	H/M	SDH (u/g)	Mb (mg/g)
trained	1970±130 (6)	49±4 (6)	360±50 (6)	0.31±0.05 (6)	0.58±0.04[a] (5)	1.37±0.09[a] (6)
untrained	1850±240 (4)	49±6 (4)	460±70 (4)	0.19±0.03 (4)	0.37±0.03 (3)	0.85±0.07 (4)

[a] Trained pigs significantly different from untrained, $P < 0.01$ (Students t-test).



LDH 1 2 3 4 5

%

	1	2	3	4	5
Trained	10.1	6.6	21.4	19.3	42.6
Untrained	1.9	3.0	13.2	17.7	64.2

F i g u r e 2. Isoenzymes of lactate dehydrogenase in m. gastrocnemius from a trained and an untrained pig.

exercise and the second peak at the end of the exercise. Contrary to this the trained pig showed a steady decrease in the lactic acid levels during, and 5, 15 and 45 min. after exercise to levels lower than the pre-exercise levels. The lactic acid levels at rest were in all 4 pigs below 20 mg/100 ml blood.

The myoglobin content and enzyme activities in m. gastrocnemius in 6 trained and 4 untrained pigs are given in Table 2. The succinic dehydrogenase and myoglobin content is significantly higher ($P < 0.01$) in trained pigs. The lactate dehydrogenase content is 22 % lower (not significant) in the trained pigs, and it appears from the H- and M-subunit ratios as well as from Fig. 2 that this decline is caused mainly by a decrease in the content of the muscle specific isoenzyme-LDH_5, which is composed by 4 M-subunits. The aldolase content is unaffected by training and the creatine phosphokinase content is only slightly elevated in trained pigs. The difference is, however, not significant.

DISCUSSION

In accordance with earlier investigations (*Ludvigsen* 1957, *Sybesma & Hessel-de Heer* 1967, *Van den Hende et al.* 1970, *Lannek et al.* 1973, *Lindberg et al.* 1973b) running exercise in untrained pigs results in increased blood lactic acid levels. The biphasic increase observed during ergometer exercise may partly be explained by the fact that muscular activity initially is performed anaerobically partly because a pure aerobic energy metabolism did not suffice to maintain muscular activity. The observed initial increase could also arise from a sudden excitement during the transport of the pig from the pen to the ergometer.

In trained pigs the blood lactic acid level did not show any increase during and following exercise. This agrees well with previous findings that the blood lactic acid level in ergometer-trained pigs is lower than that of untrained pigs following exercise (*Lannek et al., Lindberg et al.* 1973b).

In man the increase in blood lactic acid levels appears in connection with exercise carried out at an O_2 uptake corresponding to 60—70 % of the maximal O_2 uptake or above that level i.e. at conditions at which the aerobic metabolism does not suffice to cover the energy demand (*Christensen & Hansen* 1939, *Hermansen & Stensvold* 1972). Physical conditioning results in an elevation of the maximal O_2 uptake and a reduced lactic acid elevation in the blood during exercise, initially at the same absolute O_2 uptake as in untrained individuals, later also at the same relative O_2 uptake (*Ekblom* 1969, *Saltin et al.* 1969, *Hermansen* 1971). The results of this investigation thus indicate that ergometer-training of pigs as in man increases the maximal O_2 uptake thereby allowing similar exercise to be performed at a lower relative O_2 uptake in trained than in untrained pigs. Besides, the results provide evidence that the running exercise in the trained pigs has been of a pure aerobic type.

Ergometer-training results in an elevation of the succinic dehydrogenase and myoglobin content in m. gastrocnemius. A similar elevation of enzymes involved in the Krebs cycle and the respiratory chain has been noted in particular in rat skeletal muscles following an intense training program (*Holloszy* 1967, *Holloszy et al.* 1970, *Barnard & Peter* 1971, *Gollnick et al.* 1971, *Dohm et al.* 1973). The elevated enzyme levels were reported to be caused both by a proliferation and by a hypertrophy of the mitochondria in the muscle tissue (*Barnard et al.* 1970, *Gollnick et al.*). Ergometer-training of rats also results in an elevation of the myoglobin content of the skeletal muscles (*Lawrie* 1953, *Pattengale & Holloszy* 1967). Histologically these changes appear as an increase in the number of oxidative muscle fibers and a reduction of glycolytic fibers within the individual muscle (*Barnard et al.*). At the same time an increase in the density of the capillaries has been observed (*Petrén et al.* 1936, *Cotter et al.* 1973). Altogether these changes imply an increased maximal oxygen supply, an accelerated oxygen transport via myoglobin (survey by *Wittenberg* 1970) and an increase in the maximal aerobic metabolism in trained muscles.

The effect of ergometer-training on the activity of the glycolytic enzymes, lactate dehydrogenase and fructose-1,6-diphosphate aldolase in m. gastrocnemius was not unequivocal nor was it significant. Running training does not influence the aldolase level, whereas the lactate dehydrogenase content tends towards a reduction in the trained muscles. This fall is accompanied by an increase in the H/M subunit ratio, mainly due to a decrease in the muscle specific isoenzyme LDH_5. A similar finding has been reported in rats (*Novosadová* 1969). The observed shift in the H- and M-subunit distribution supports the hypothesis about a change in the muscle cell metabolism towards an aerobic type during training, as it has been shown that an increase in the intracellular oxygen tension results in an elevated synthesis of H-subunits in vitro, whereas low oxygen levels favour the synthesis of M-subunits (*Hyldgaard-Jensen* 1971).

Information concerning the effect of training on the CPK content in muscle tissue is scarce. In accordance with the findings reported here *Rawlinson & Gould* (1959) could not demonstrate an effect of training on the CPK content in rat muscles. *Wagner & Critz* (1970) on the contrary noted that the CPK content in trained rats was higher than in untrained rats. The difference found between trained and untrained rats might be explained by the fact that the untrained animals in the work referred to had been subjected to a limited freedom of movement, which is known to produce a decrease in the muscle tissue CPK content (*Kendrick-Jones & Perry* 1965).

Skeletal muscles from ergometer-trained pigs have a higher content of adenosin triphosphate (ATP) and a reduced rate of glycolysis post mortem compared to that of untrained animals (*Rülcker* 1968, *Lindberg et al.* 1973a). Furthermore the respiratory rate and consequently the aerobic ATP production are higher in mitochondria isolated from muscle tissue of pigs possessing a low post-mortem glycolytic rate (*Eikelenboom* 1972, *Eikelenboom & van den Bergh* 1973). Taken together with results of this investigation evidence is presented that ergometer-training of pigs results in a changed muscle metabolism towards an aerobic type during exercise and in periods with an increased demand for energy. Hereby the aerobic ATP production is enhanced at the expense of the ATP production from anaerobic glycolysis and glycogenolysis, and consequently the lactic acid accumulation in the muscle tissue is reduced.

Provided this difference in the muscle metabolism type, as it is expressed by the individual differences in its enzyme composition, is present in a population of untrained pigs, a determination of the muscle enzyme composition might be a useful tool in the in vivo evaluation of the post-mortem rate of glycolysis and for a prediction of animals whose skeletal muscles exhibit PSE changes post mortem.

ACKNOWLEDGEMENT

The authors wish to address their gratitude to Professor, dr. med. vet. J. Moustgaard on whose initiative the present investigation was undertaken. For valuable assistance during the work we wish to thank dr. med. vet. Birthe Palludan as well as S. Blirup-Jensen, D. V. M.

REFERENCES

Barker, S. B. & W. H. Summerson: The colorimetric determination of lactic acid in biological material. J. biol. Chem. 1941, *138*, 535—554.

Barnard, R. J. & J. B. Peter: Effect of exercise on skeletal muscle. III. Cytochrome changes. J. appl. Physiol. 1971, *31*, 904—908.

Barnard, R. J., V. R. Edgerton & J. B. Peter: Effect of exercise on skeletal muscle. I. Biochemical and histochemical properties. J. appl. Physiol. 1970, *28*, 762—766.

Bickhardt, K., H.-J. Chevalier, W. Giese & H.-J. Reinhard: Akute Rückenmuskelnekrose und Belastungsmyopathie beim Schwein. (Acute back muscle necrosis and exertional myopathy in pigs). Fortschritte der Veterinärmedizin. Vol. 18. Paul Parey, Berlin 1972.

Christensen, E. H. & O. Hansen: I. Zur Methodik der respiratorischen Quotient-Bestimmungen in Ruhe und bei Arbeit. II. Untersuchungen über die Verbrennungsvorgänge bei langdauernder, schwerer Muskelarbeit. III. Arbeitsfähigkeit und Ernährung. IV. Hypoglykämie, Arbeitsfähigkeit und Ermüdung. V. Respiratorischer Quotient und O_2-Aufnahme. (I. Methods for the determination of the respiratory quotient during rest and exercise. II. Investigations of metabolic pathways during prolonged, heavy exercise. III. Performance and nutrition. IV. Hypoglycemia, performance and fatique. V. The respiratory quotient and O_2-uptake). Skand. Arch. Physiol. 1939, *81*, 137—189.

Cotter, M., O. Hudlická & G. Vrbová: Growth of capillaries during long-term activity in skeletal muscle. Bibl. anat. (Basel) 1973, no. 11, 395—398.

Dohm, G. L., R. L. Huston, E. W. Askew & H. L. Fleshood: Effects of exercise, training and diet on muscle citric acid cycle enzyme activity. Canad. J. Biochem. 1973, *51*, 849—854.

Eikelenboom, G.: Stress-susceptibility in swine and its relationship with energy metabolism in skeletal musculature. Thesis. Res. Inst. Animal Husbandry „Schoonoord", Zeist, Netherlands 1972.

Eikelenboom, G. & S. G. van den Bergh: Mitochondrial metabolism in stress-susceptible pigs. J. Animal Sci. 1973, *37*, 692—696.

Ekblom, B.: Effect of physical training on oxygen transport system in man. Acta physiol. scand. 1969, suppl. 328.

Gollnick, P. D., C. D. Ianuzzo & D. W. King: Ultrastructural and enzyme changes in muscles with exercise. *In* Muscle Metabolism during Exercise, 69—81. Eds. B. Pernow & B. Saltin, Plenum Press, New York 1971.

Hermansen, L.: Lactate production during exercise. *In* Muscle Metabolism during Exercise, 401—407. Eds. B. Pernow & B. Saltin, Plenum Press, New York 1971.

Hermansen, L. & I. Stensvold: Production and removal of lactate during exercise in man. Acta physiol. scand. 1972, *86*, 191—201.

Holloszy, J. O.: Biochemical adaptations in muscle. Effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. J. biol. Chem. 1967, *242*, 2278—2282.

Holloszy, J. O., L. B. Oscai, I. J. Don & P. A. Molé: Mitochondrial citric acid cycle and related enzymes: Adaptive response to exercise. Biochem. biophys. Res. Commun. 1970, *40*, 1368—1373.

Hyldgaard-Jensen, J. F.: Lactate dehydrogenase in pigs. Studies on lactate dehydrogenase isoenzymes in blood and organs. Thesis. Munksgaard, Copenhagen 1971.

Jørgensen, P. F.: Muskelenzymer hos svin. Studier over blodets og muskelvævets enzymindhold hos normale og ergometertrænede grise. (Muscle enzymes in pigs. Studies on enzymes in blood and muscle tissue in normal and ergometer-trained pigs). Licentiatafhandling. The Royal Veterinary and Agricultural University, Copenhagen 1974.

Kendrick-Jones, J. & S. V. Perry: Enzymatic adaptation to contractile activity in skeletal muscle. Nature (Lond.) 1965, *208*, 1068—1070.

Lannek, N., P. Lindberg, L. Jönsson & G. Johansson: Blocked utilization of oxygen in pigs during exercise. Acta vet. scand. 1973, *14*, 492—494.

Lawrie, R. A.: Effect of enforced exercise on myoglobin concentration in muscle. Nature (Lond.) 1953, *171*, 1069—1070.

Lindberg, P., N. Lannek & L. Blomgren: The influence of physical training on the pH of skeletal muscle in pigs. Acta vet. scand. 1973a, *14*, 359—365.

Lindberg, P., N. Lannek, L. Blomgren, G. Johansson & L. Jönsson: Blood lactic acid in untrained and trained pigs under stress conditions. Nord. Vet.-Med. 1973b, *25*, 619—626.

Ludvigsen, J. B.: Akuter Herztod und Skelettmuskelentartung des Schweines. (Acute "Herztod" and skeletal muscle degeneration in pig). Arch. exp. Vet.-Med. 1957, *11*, 198—224.

Mount, L. E. & D. L. Ingram: The Pig as a Laboratory Animal. Acad. Press, London 1971.

Novosadová, J.: Lactic dehydrogenase isoenzymes in serum and tissues after exercise in rats. *In* Medicine and Sport. Vol. 3. Biochemistry of exercise, 239—244. Ed. J. R. Poortmans, S. Karger, Basel-New York 1969.

Palludan, B.: A-avitaminosis in swine. A study on the importance of vitamin A for reproduction. Thesis. Munksgaard, Copenhagen 1966.

Pattengale, P. K. & J. O. Holloszy: Augmentation of skeletal muscle myoglobin by a program of treadmill running. Amer. J. Physiol. 1967, *213*, 783—785.

Petrén, T., T. Sjöstrand & B. Sylvén: Der Einfluss des Trainings auf die Häufigkeit der Capillaren in Herz- und Skelettmuskulatur. (Effect of training on the density of capillaries in heart and skeletal muscle). Arbeitsphysiologie 1936, *9*, 376—386.

Rawlinson, W. A. & M. K. Gould: Biochemical adaptation as a response to exercise. 2. Adenosine triphosphatase and creatine phosphokinase activity in muscles of exercised rats. Biochem. J. 1959, *73*, 44—48.

Rülcker, C.: The influence of physical training and short-time physical stress on colour, fluid loss, pH, adenosine triphosphate and glycogen of the gracilis muscle in pigs. Acta vet. scand. 1968, suppl. 24.

Saltin, B., L. H. Hartley, Å. Kilbom & I. Åstrand: Physical training in sedentary middle-aged and older men. II. Oxygen uptake, heart rate, and blood lactate concentration at submaximal and maximal exercise. Scand. J. clin. Lab. Invest. 1969, *24*, 323—334.

Sybesma, W. & J. C. M. Hessel-de Heer: LDH_5 and meat quality in pigs. 13th Europ. Meet. Meat Res. Workers, Rotterdam 1967.

Van den Hende, C., E. Muylle & W. Oyaert: Oxygen utilization and metabolic acidosis after exercise in pigs. Zbl. Vet.-Med. A 1970, *17*, 167—173.

Wagner, J. A. & J. B. Critz: The effect of physical activity on creatine phosphokinase and glutamic-oxalacetic transaminase levels in muscle and blood plasma of rats. Physiologist 1970, *13*, 332.

Wittenberg, J. B.: Myoglobin-facilitated oxygen diffusion: Role of myoglobin in oxygen entry into muscle. Physiol. Rev. 1970, *50*, 559—636.

SAMMENDRAG

Effekt af fysisk træning på skeletmuskulaturens enzymsammensætning hos grise.

Muskelarbejde hos grise medfører en stigning i blodets mælkesyreindhold. Denne stigning iagttages ikke hos i forvejen trænede dyr.

Indholdet af enzymerne ravsyredehydrogenase, fruktose-1,6-difosfat aldolase, mælkesyredehydrogenase og kreatinfosfokinase samt indholdet af myoglobin er bestemt i m. gastrocnemius fra 6 ergometertrænede og 4 utrænede grise. Ravsyredehydrogenase- og myoglobinindholdet er signifikant højere ($P < 0,01$) hos trænede dyr, medens der ikke er iagttaget ændringer i indholdet af fruktose-1,6-difosfat aldolase og kreatinfosfokinase. Mælkesyredehydrogenaseindholdet tenderer mod et fald hos de trænede dyr og er ledsaget af en stigning i forholdet mellem H- og M-subunits af mælkesyrehydrogenase.

Resultaterne peger i retning mod en stigning i muskulaturens maksimale aerobe stofskifte hos trænede dyr og tyder på, at trænede grise i højere grad end utrænede benytter det aerobe energistofskifte under muskelarbejde.

(Received March 4, 1975).

Reprints may be requested from: P. Fogd Jørgensen, Department of Physiology, Endocrinology and Bloodgrouping, Royal Veterinary and Agricultural University, Bülowsvej 13, DK-1870 Copenhagen V, Denmark.