INFLUENCE OF PREDNISOLONE OR CORTISOL INJECTION ON MUSCLE GLYCOGENOLYSIS DURING TRANSPORT STRESS IN LAMBS

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INTRODUCTION

Glycogen metabolism is of great importance for meat quality of domestic animals, since the level of muscle glycogen at slaughter determines ultimate meat pH (see LAWRIE, 1966). To a large extent preslaughter stress influences muscle glycogen level (LAWRIE, 1966). On the other hand, adrenal function, known to affect muscle glycogen metabolism, is largely implicated in reaction to stress. In the pig, adrenal function, as related to pale, soft, exsudative muscle, has been studied extensively in work on meat quality defects (for a review, see CASSENS et al., 1975). In contrast, little work has been devoted to influence of adrenal function on meat quality in sheep or cattle. HEDRICK et al. (1959), then BENDALL and LAWRIE (1962) (the latter cited by LAWRIE, 1966) showed that cortisone administration largely prevented both the glycogen-depleting effect and consequent meat pH increase due to adrenaline injections, when corticosteroid was given about 24 hours before adrenaline. LAWRIE (1966) concluded that DFD meat in cattle might be due partly to an inability of the adrenal cortex to counteract a prolonged adrenaline hypersecretion. More recently, JEDICKLA et al. (1979) observed a relationship between blood level of cortisol and meat pH-value in bulls handled for slaughter. Results from experiments designed to investigate the influence of cortisol or of induced adrenal insufficiency on glycogen metabolism and ultimate meat pH in lambs transported before slaughter are presented.

MATERIAL AND METHODS

1 - Animals and experimental design

26 male Limousin lambs, aged six months, were bought from one farm about 4 weeks before the experiment and then fed a concentrate (cereals and pelleted hay - about 0.5 kg per animal per day) and hay (ad libitum). All the lambs were kept in one pen. In experiment 1, 20 lambs were allotted to four experimental groups with 5 lambs in each. An attempt was made to distribute genetic origin (ram) between experimental groups. For eight days, 5 lambs received subcutaneously a daily injection of methylprednisolone hemisuccinate (Solumédrol, Upjohn Laboratories) (1 mg per kg of liveweight). Injections were made between 9 and 10 a.m. Methylprednisolone was injected as solution in water at a concentration of 10 mg per ml. At the same time, 5 animals were injected subcutaneously with 0.1 ml per kg of liveweight of 8°/$_{oo}$ saline (in order to obtain a similar volume of injection as in the case of methylprednisolone). The 10 remaining animals received no treatment. On the eighth day, after the last injections, all the animals were brought to the meat laboratory (about 1 km away) and fitted with a polyethylene catheter (length : 60 mm ; diameter 2 mm) in the jugular vein, to facilitate blood sampling and intravenous injections. Animals were kept in one pen at the meat laboratory until the following morning, when blood was taken from each animal through the catheter. 5 animals (3 had been previously injected with saline and 2 not injected) were slaughtered without further treatment (between 10 and 11 a.m.). Of the 15 remaining lambs, 5 received a dose of 50 mg of cortisol (about 1.25 mg/kg liveweight) through the catheter. Then the 15 lambs were transported by truck. After two hours of transport, a 30 minutes stop allowed blood sampling of each animal to take place, then the ride resumed. Transport plus stop lasted exactly four hours. Blood was again taken from each animal at the end of the trip. The lambs were then slaughtered within 90 minutes after treatment between 2 and 3.30 p.m., alternating animals from each of the three treatment groups.

Animals which were not transported before slaughter were referred to as "control". Transported animals were referred to as "cortisol treated", "prednisolone treated" or "transported control" according to the treatment they had experienced before transport. The "transported control" group was made up of 2 lambs which had been injected with saline and 3 which had never been injected before transport treatment.

An effect of methylprednisolone treatment on muscle glycogen level at rest was expected. So a group of lambs injected with methylprednisolone but not transported could have been included, to estimate the effect of transport stress in such animals more accurately by comparison with methylprednisolone treated and transported ("prediusolone treated" group). This was impossible due to the large number of lambs already involved in experiment 1. We thus designed a second experiment to estimate the effect of the methylprednisolone treatment on muscle glycogen level at rest. 6 lambs were allotted to 2 experimental groups. 3 lambs were injected with methylprednisolone and 3 with saline for eight days in exactly the same conditions described previously. They were then treated as animals in experiment 1 (transport to meat laboratory, catheterization). The day following the last injections and catheterization, blood was taken from each lamb through the catheter, then the animals were slaughtered.

2 - Slaughter, muscle sampling and analytical techniques

Animals were killed by severing all major neck veins and arteries. Between 15 and 20 minutes after slaughter, samples were taken from Rectus abdominis (RA), Pectoralis profundus (PP), Semitendisosus (ST) and Supraspinatus (SS) muscles and from the liver (in three standardized locations) and were put into liquid nitrogen and kept frozen (- 20° C) for subsequent glycogen and lactic acid determinations. 60 minutes after slaughter, carcasses were put into a + 10° C room. 24 to 26 hours later, pH was measured using a Radiometer 29 pH meter and inserting the probe electrode into the intact muscle tissue on the Longissimus dorsi (LD), Adductor (A), Semimembranosus (SM), Biceps femoris (BF), Triceps brachii (TB), Psoas major (PM), Infraspinatus (IS), SS, PP, ST and RA muscles. The pH value obtained was referred to as ultimate pH. Frozen samples were ground in a Waring blendor (for liver, the three sam-

ples were mixed). The freezing was maintained during grinding with liquid nitrogen. 2 g of muscle powder were homogeneized in 18 ml 0.005 M sodium iodoacetate and pH was measured in this homogenate using a Radiometer 29 pH meter. 5 g of muscle powder were homogeneized in 25 ml 0.6 M cold perchloric acid. Glycogen was determined in aliquots of this homogenate according to the technique of DALRYMPLE and HAMM (1973) with minor modification. Homogenate was neutralized by 3 M K_2CO_3 filtered, and lactic acid was determined in the filtrate (HOHORST, 1963). To correct glycogen values for the loss due to post mortem glycogenolysis between death and sampling time, lactic acid level was added to the glycogen level as measured at sampling, and the sum referred to as "muscle glycogen" (GIRE and MONIN, 1979). Liver glycogen was determined in a homogenate of 5 g of liver powder in 25 ml 0.6 M perchloric acid, using the technique of DALRYMPLE and HAMM (1973) with minor modification.

Blood samples were divided into two parts just after they had been obtained. One part was immediately deproteinized by adding 2 volumes of 0.6 M cold perchloric acid and then contrifuging. Lactic acid (HOHORST, 1963) and glucose (TRINDER, 1969) were measured in the supernatant. The other part was placed in ice for less than 30 minutes, then centrifuged in a cold room (+ 4° C). Cortisol was measured in the plasma (according to the technique of MURPHY (1967), as slightly modified by GIRE, 1976).

RESULTS AND DISCUSSION

Since we found no differences in blood composition, muscle glycogen level or ultimate meat pH between animals injected with saline or not injected at all, the two types of lambs were put together for calculations, labeled "control" or "transported control" without further distinction.

Methylprednisolone injections depressed adrenal function to some extent. We found a reduction in adrenal gland weight in the "prednisolone-treated" animals as opposed to "controls" (- 38 %, $P < 0.05$, for the left adrenal gland ; - 14 %, non-significant, for the right one). Blood cortisol level was reduced in "prednisolone-treated" animals at rest (3.0 ng/ml blood vs 10.7 in "control", $P < 0.01$) but not during stress (18.5 ng/ml blood vs 21.1 in "control" after 2 hours of transport, non significant difference ; 12.1 ng/ml blood vs 18.2 in "control" after 4 hours, non significant difference). Cortisol injection increased blood cortisol level very sharply (102 ng/ml blood in "cortisol treated" vs 21 in "control", $P < 0.01$, after 2 hours of transport ; 29 ng/ml blood vs 18 in control, $P < 0.05$, after 4 hours). Methylprednisolone treatment increased glycogen level in muscles (+ 44 %, $P < 0.01$, in PP ; + 45 %, $P < 0.01$, in ST ; + 31 %, $P < 0.01$, in SS) and in liver (+ 102 %, $P < 0.01$) as shown in figure 1.

Blood glucose or lactic acid levels were not significantly changed by transport stress alone, as shown in figure 2. Conversely, higher blood glucose levels were observed after 2 hour transport among the "cortisol treated" ($P < 0.05$) or "prednisolone-treated" ($P < 0.05$), and after 4 hour transport among the "cortisol treated" ($P < 0.05$). Similarly lactic acid blood level increased after 4 hours in "cortisol treated" animals ($P < 0.01$) and after 2 or 4 hour transport in "prednisolone-treated" ($P < 0.05$).

Results of muscle glycogen, liver glycogen and pH measurements are shown in figure 3. Transport stress alone decreased glycogen level only in SS muscle of "transported control" animals. The decrease due to stress was greater in animals treated by hormones : there were differences in RA, ST and PP muscles between "control" and "prednisolone treated" animals ($P < 0.05$ in the three muscles), and in RA, ST, PP and SS muscles between "control" and "cortisol treated" ($P < 0.05$ in the four muscles). Compared to "transported control", "cortisol treated" had a lower glycogen level in all four muscles studied ($P < 0.05$), but no difference was found in any muscle of "prednisolone treated". However, glycogen mobilization was obviously higher in "prednisolone treated" than in "transported control", since methylprednisolone treatment increases muscle glycogen at rest to a great extent as previously mentioned (figure 1).

Transport stress alone increased ultimate pH in LD, RA, ST and SM muscles ($P < 0.05$ in the four muscles) as indicated by the comparison of "control" and "transported control" (figure 3). "Cortisol treated" lambs had a higher pH in RA ($P < 0.05$) and PP ($P < 0.05$) muscle than "transported control". In contrast to "transported control", the pH was similar in all muscles in "prednisolone treated" animals. However, as already indicated, this observation does not exclude a more intensive mobilization during preslaughter stress in "prednisolone treated" lambs.

Liver glycogen was higher in "cortisol treated" and in "prednisolone treated" lambs than in either "control" or "transported control" animals (figure 3).

The results indicate that cortisol or methylprednisolone treatments increased muscular glycogen mobilization during transport stress. The increased blood glucose level in "cortisol treated" animals might be due to a stimulation of neoglucogenesis by cortisol. However, the increase of both blood lactic acid and muscle glycogen mobilization in these animals indicated that cortisol enhanced the glycogenolytic effect of stress. LABORIT (1976) found that ACTH administration enhanced adrenaline release in rats. Thus, in our experiment, treatment with a high dose of cortisol might have provoked a hypersecretion of adrenaline and subsequently a higher muscle glycogenolysis. In any case, high doses of cortisol do not prevent blood lactic acid increase in lambs conversely to what LUDVIGSEN (1957) noted in pigs. LUDVIGSEN (1969) also reported that corticosteroid injection protected stress-susceptible pigs against adverse effects of thermal stress. Such a protective effect was not observed in lambs experiencing transport stress ; on the contrary we noted an enhancement of the stress effect, in terms of muscle glycogenolysis. On the other hand, adrenal impairment brought on by methylprednisolone treatment also increased blood lactic acid level and muscle glycogen mobilization during the transport stress. It appears that adrenal balance can be important to allow the lambs to react properly to stress, and to avoid adverse meat quality changes when stress before slaughter occurs.

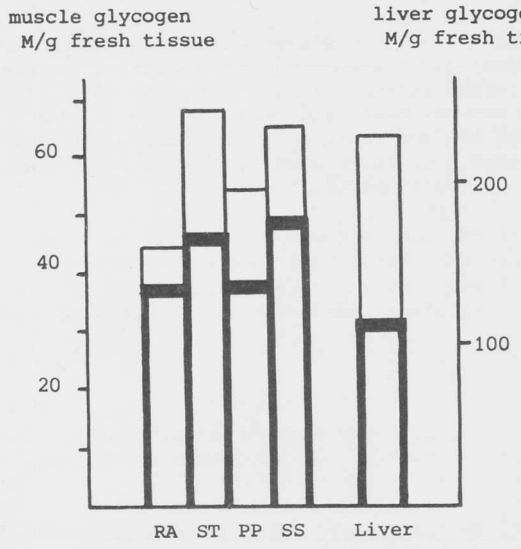

Figure 1 : INFLUENCE OF METHYLPREDNISOLONE TREATMENT ON GLYCOGEN LEVEL IN FOUR MUSCLES AND LIVER (n = 3 lambs in each group)

▬ saline injected

—— methylprednisolone injected

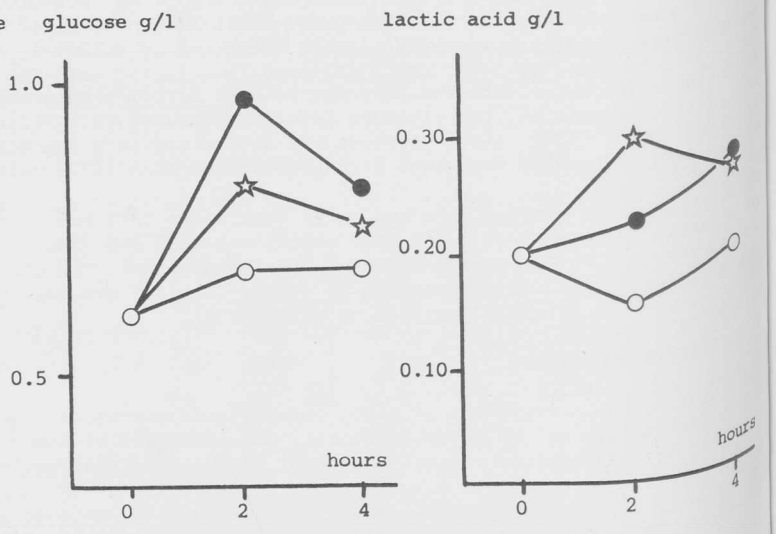

Figure 2 : INFLUENCE OF METHYLPREDNISOLONE TREATMENT OR CORTISOL INJECTION ON BLOOD GLUCOSE AND LACTIC ACID CHANGES DURING TRANSPORT STRESS IN LAMBS (n = 5 lambs in each group)

○ "transported control"

☆ "prednisolone treated"

● "cortisol treated"

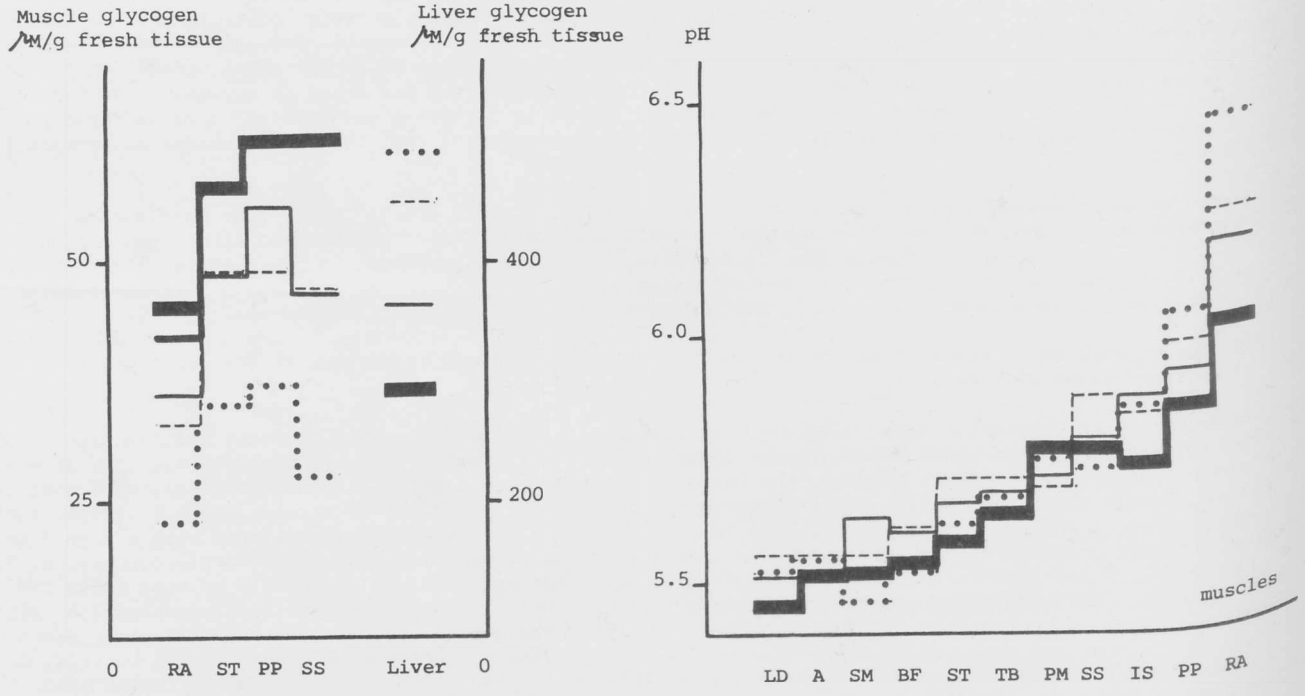

Figure 3 : INFLUENCE OF METHYLPREDNISOLONE TREATMENT OR CORTISOL INJECTION ON CHANGES IN MUSCLE GLYCOGEN AND MEAT pH DUE TO TRANSPORT STRESS IN LAMBS (n = 5 lambs in each group)

▬ "control" —— "transported control" ••••• "cortisol treated"

------ "prednisolone treated"

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