EVOLUTION OF GLYCOGEN LEVEL, PHOSPHORYLASE ACTIVITY AND GLYCOGEN SYNTHETASE ACTIVITY IN VARIOUS LAMB MUSCLES DURING GROWTH

A. TALMANT [x], Marièle BRIAND [xx], G. MONIN [x], R. DURAND [x]

[x] Institut National de la Recherche Agronomique, Theix, 63110 BEAUMONT, France
[xx] Université de Clermont 2, les Cézeaux, 63170 AUBIERE, France

INTRODUCTION

DFD meat results of a lack of glycogen in the muscles of animals experiencing prolonged stress (as transport for instance) before slaughter (LAWRIE, 1966). MONIN and GIRE (1977) showed that in sheep the mobilization of muscle glycogen during transport stress increases with age, this phenomenon leading to a more pronounced increase of meat pH after slaughter in older animals (between 6 to 10 months). Moreover, susceptibility to preslaughter stress, in terms of DFD meat occurence, varies largely between muscles in the sheep (GIRE and MONIN, 1979), as also reported in cattle by TARRANT (1976) and SORNAY and LEGRAS (1978). This inter-muscle variation could be due to differences in glycogen levels or in glycogen metabolism. Such differences are known to be related to the metabolic type of muscle (BEATTY et al., 1963 ; BOCEK et al., 1966). So it seemed to us of interest to look at the changes in the glycogen levels and in the main enzymes regulating glycogen metabolism, i.e. glycogen synthetase and glycogen phosphorylase, during growth in muscles differing by their metabolic characteristics in lambs.

MATERIAL AND METHODS

Two experiments were designed, using male crossbred Ile de France x (Limousine x Romanov) lambs. All the animals were kept inside for the experiment. After weaning (at about 60 days of age), they were fed a cereal concentrate (about 0.5 kg per animal per day) and hay or straw ad libitum. In the first experiment, eight animals were killed at an average of 220 ± 7 days (mean ± s.e.m.). In the second one, 25 lambs were alloted to five groups of five animals in each. The groups were slaughtered at respective average ages of 72 ± 2, 99 ± 2, 191 ± 5, 355 ± 5 and 395 ± 7 days.

Between 10 and 20 minutes after exsanguination, samples were obtained of Longissimus dorsi (LD), Adductor (A), Tensor fasciae latae (TFL), Supraspinatus (SS) muscles and of the white portion of Semitendinosus (ST) in first experiment. In the second experiment Semitendisosus was replaced by heart (left ventricle). Samples were carefully trimmed of fat and connective tissue and divided into five parts. One part was homogeneized in 20 volumes of an extraction medium containing glycylglycine (63 mM), saccharose (500 mM), EDTA (6.2 mM), NaF (125 mM) dithiotriethol (5 mM) and adjusted to pH 7.4, for glycogen synthetase and glycogen phosphorylase determinations.

Another part was homogeneized in 5 volumes of a medium containing potassium oxalate (5 mM), imidazole (20 mM) and potassium chloride (80 mM), for extraction of myofibrils and measurement of myofibrillar ATPase activity. Other parts were used for oxidative capacity measurements (results not reported in detail here) and haeminic iron content. The last part was put into liquid nitrogen and kept frozen (- 20° C) for subsequent glycogen and lactic acid determinations.

Glycogen phosphorylase (a + b) activity was determined in the presence of AMP by the technique of WANG and ESMANN (1972) and glycogensynthetase (I + D) in the presence of glucose-6-phosphate by the technique of THOMAS et al. (1968), with minor modifications (PERET, personal communication). Myofibrillar ATPase activity was measured in the presence of Ca ++ and Mg ++, according to the technique described by GOODNO et al. (1979). Protein contents of the whole muscle homogenate and of the myofibrils suspension were determined using the biuret method. Haeminic iron was measured according to the technique of HORNSEY (1956) ; this technique measures the whole haeminic iron content of the muscle composed of approximately 90 to 95 % myoglobin and 5 to 10 % other pigments. Glycogen was determined according to the technique of DALRYMPLE and HAMM (1973) as slightly modified by GIRE (1976), in a homogenate of 5 g muscle in 25 ml of 0.6 M perchloric acid ; lactic acid was determined in the same homogenate after neutralization by 3 M K_2CO_3 according to the technique of HOHORST (1963). In an attempt to correct for the post mortem degradation of glycogen, glycogen level in vivo was estimated by adding glycogen and lactic acid contents (GIRE and MONIN, 1979).

Results were expressed as follows : glycogen synthetase and glycogen phosphorylase in μm glucose/mn/g muscle protein (whole homogenate) ; ATPase in μM KOH/mn/g myofibrillar protein ; glycogen in μM glucose/g fresh tissue ; haeminic iron in μg/g fresh tissue ; in experiment 1, glycogen synthetase and phosphorylase in μM glucose/mn/g muscle.

RESULTS AND DISCUSSION

It was possible to distinguish three groups among the six skeletal muscles under study, as shown in fig. 1. TFL and ST had a high ATPase activity and a low myoglobin content, the latter trait indicating a low oxidative capacity as shown by LAWRIE (1952) (close relationships between haeminic iron level and some activities of mitochondrial enzymes (iron - cytochrome oxydase : r = 0.89 ; iron - succinate dehydrogenase : r = 0.79) in the lambs considered in this study could be assessed). This indicates that these muscles are predominantly composed of "fast twitch-glycolytic" myofibers, as reported by PETER et al. (1972) (αw myofibers according to the classification of ASHMORE and DOERR, 1971). High ATPase and low haeminic iron content of LD and A muscles indicate that these muscles are predominantly composed of "fast-twitch-glycolytic-oxidative" fibers (PETER et al., 1972 ; αR myofibers of ASHMORE and DOERR). Low ATPase and mean haeminic iron level of SS and TB muscles are characteristic of muscles predominantly composed of "slow-twitch-oxidative" (PETER et al.) or βR (ASHMORE and DOERR) myofibers.

These biochemical results agree well with histochemical observations of LACOURT (1974) for LD and TFL muscles of lamb. For simplicity, in this text, we will call ST and TFL "fast white" muscles, LD and A "fast red" muscles and SS and TB "slow red" muscles, although probably none of these muscles is composed of a single type of myofibers.

Between 2 and 13 months, the levels of ATPase activity did not change significantly, but the haeminic iron content did. However, the relative positions of the points representing the five muscles studied in experiment 2 on the graph were approximately kept the same as shown in fig. 2. So we considered that the classification into "fast white", "fast red" or "slow red" of the various muscles under study remained valid during the growth of the lambs.

Table 1 shows activities of glycogen phosphorylase and glycogen synthetase, and glycogen level in the six skeletal muscles studied in experiment 1. Glycogen synthetase was the highest in the "fast red" LD and A muscles, and the lowest in the "slow red" SS and TB, the "fast white" ST and TFL muscles having intermediate values. PETTE and DOLKEN (1975) also reported a higher glycogen synthetase activity in "fast red" than in "fast white" or "slow red" muscles of the guinea pig. Previously BOCEK and BEATTY (1966) and JEFRESS et al. (1968) found higher glycogen synthetase activity in red than in white muscles, but they did not distinguish between "fast" and "slow" red muscles. Phosphorylase activity was higher in "fast" (red or white) LD, A, TFL and ST muscles than in "slow" TB and SS muscles . This agrees with the results of PETER et al. (1972) as well as of PETTE and DOLKEN (1975) in the guinea-pig.

Glycogen level was higher in "fast red" LD and A muscles than in either "slow red" TB and SS or "fast white" TFL and ST muscles. In the guinea-pig, PETER et al. (1972) found also the highest glycogen level in the "fast red" red portion of the muscle vastus lateralis (9.7 mg/g wet weight), but they observed a large difference between the "fast white" white portion of the vastus lateralis (7.4 mg/g) and the "slow red" soleus muscle (3.3 mg/g). It is noteworthy that the range of glycogen values found by these authors in the guinea-pig was much more important than that we observed in the lamb. OGATA (1960) in the rabbit, BOCEK et al. (1963) in the rat reported glycogen content to be lower in red than in white muscle ; BEECHER et al. (1965) found few differences in glycogen content of various porcine muscles differing by their red fiber content ; this discrepancy with our results can be explained by the fact that they did not make a distinction between "fast red" and "slow red" muscles.

During growth from 2 to 13 months, glycogen synthetase and glycogen phosphorylase activities decreased in the heart and the five skeletal muscles under investigation ($P < 0.05$ to $P < 0.01$). The decrease seemed to be faster between 2 and 6 months of age, however in view of the rather small number of experimental points it was not possible to ascertain differences in this respect between the different phases of the growth. Glycogen synthetase decreased faster in the "fast red" LD and A muscles than in the heart or the other skeletal muscles. Glycogen content decreased quite regularly in all the muscles between 2 and 13 months of age. This result confirms previous observations of MONIN and GIRE (1977). These results are shown in figures 3 and 4, and in table 2.

GIRE and MONIN (1979) reported that among eleven muscles that they studied, LD and A muscles had the lowest ultimate pH in animals killed at rest, as well as in animals killed after stress. Conversely SS and TB had a higher pH at rest, and experienced a larger increase after stress ; ST showed intermediate behaviour in these two respects. So it might be a rather complex relationship between muscle metabolic type and meat ultimate pH, "fast red" muscles showing the lowest ultimate pH and "slow red" muscles the highest one.

This relationship could be partly explain by the levels of glycogen in these different muscles, "fast red" having the highest and "slow red" the lowest, but this is not the only explanation since GIRE and MONIN (1979) showed that even in SS muscle residual glycogen remaining after pH fall completion could allow a further pH fall of 0.25 pH unit if completely transformed into lactic acid. Such observations had been previously made in cattle and horses by LAWRIE (1955).

The increase with age in muscle glycogen mobilization due to transport stress observed in the sheep by MONIN and GIRE (1977) cannot be explained by the changes in phosphorylase (a + b) activity. However decrease of muscle glycogen levels could contribute to the increase in meat ultimate pH with age found in lambs stressed (transported) before slaughter. This change in glycogen content of the skeletal muscles could be related to a decline in glycogen synthetase activity.

REFERENCES

1/ ASHMORE C.R., DOERR L., 1971 - Exptl. Neur., 31, 408 -- 2/ BEATTY C.H., PETERSON R.D., BOCEK R.M., 1963 - Amer. J. Physiol., 204, 939 -- 3/BEECHER G.R., CASSENS R.G., HOEKSTRA W.G., BRISKEY E.J., 1965 - J. Food Sci., 30, 969 4/ BOCEK R.M., BEATTY C.H., 1966 - J. Histochem. Cytochem., 14, 549 -- 5/ BOCEK R.M., PETERSON R.D., BEATTY C.M., 1966 - Amer. J. Physiol., 210, 1101 -- 6/ DALRYMPLE R.H., HAMM R., 1973 - J. Food Techn., 8, 439 -- 7/ GIRE P., 1976 - Thèse Doctorat de Spécialité, Université de Clermont 2, n° 505 -- 8/ GIRE P., MONIN G., 1979 - Ann. Technol. Agric., 28 (in press) -- 9/ GOODNO C.C., WALL C.M., PERRY S.V., 1978 - Biochem. J., 175, 813 -- 10/ HOHORST H.J., 1963 in methods of Enzymatic Analysis (ed. H.U. BERGMEYER) Academic Press, New-York -- 11/ HORNSEY H.C., 1956 - J. Sci. Food Agric., 7, 534 -- 12/ JEFRESS R.N., PETER J.B., LAMB D.R., 1968 - Life Sci., 7, 957 -- 13/ LACOURT A., ARNAL M., 1974 - 20th European meeting of Meat Research Workers, N 3, Dublin, 15-20 September -- 14/ LAWRIE R.A., 1952 - Nature (London), 170, 122 -- 15/ LAWRIE R.A., 1955 - Biochim. biophys. Acta, 17, 282 -- 16/ LAWRIE R.A., 1966 in Physiology and Biochemistry of muscle as a food. p 137. (eds E.J. BRISKEY, R.G. CASSENS, J.C. TRAUTMAN) Univ. Wisconsin Press, Madison -- 17/ MONIN G., GIRE P., 1977 - 23th European meeting of meat research workers B 1, Moscow, 4-9 September -- 18/ OGATA T., 1960 - J. Biochem., Tockyo, 47, 726 -- 19/ PETER J.B., BARNARD R.J., EDGERTON V.R., GILLESPIE C.A., STEMPEL K.E., 1972 - Biochemistry, 11, 2627 -- 20/ PETTER D., DOLKEN G., 1975 - Adv. Enz. Regul., 13, 355 -- 21/ THOMAS J., SCHLENDER K., LARNER J., 1968 - Anal. Biochem., 25, 486 -- 22/ SORNAY J., LEGRAS P., 1978 - Ind. Alim. Agric., 4, 392 -- 23/ TARRANT P.J., 1976 - 24th European meeting of meat research workers, B 8, Malmö, 30 August-3 September -- 24/ WANG P., ESMANN V., 1972 - Anal. Biochem., 25, 486

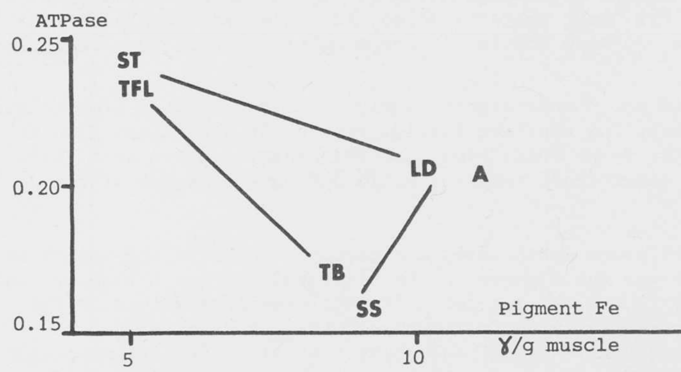


Figure 1 : METABOLIC TYPE OF SOME LAMB MUSCLES AS INDICATED BY ATPase ACTIVITY AND PIGMENT IRON CONTENT

ST : Semitendinosus ; TFL : Tensor fasciae latae ; LD : Longissimus dorsi ; A : Adductor ; TB : Triceps brachii ; SS : Supraspinatus

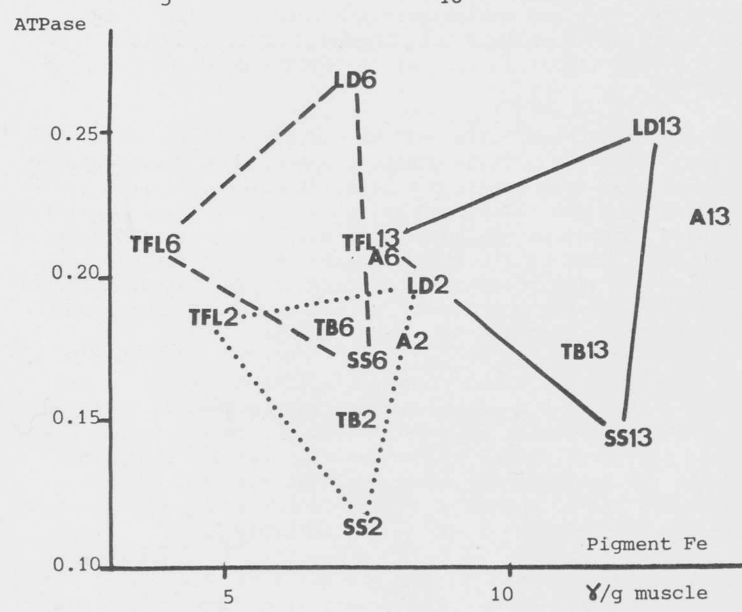
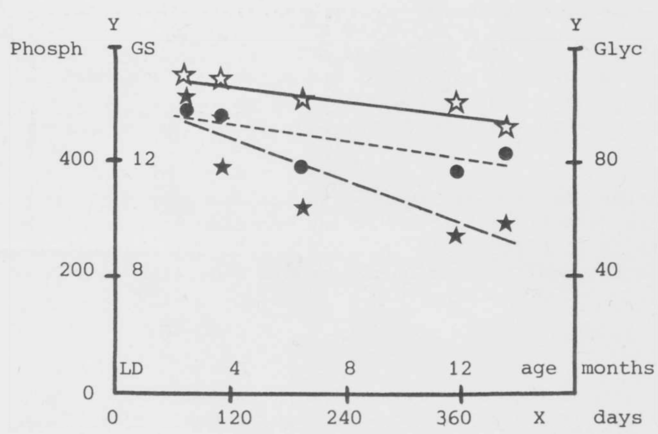
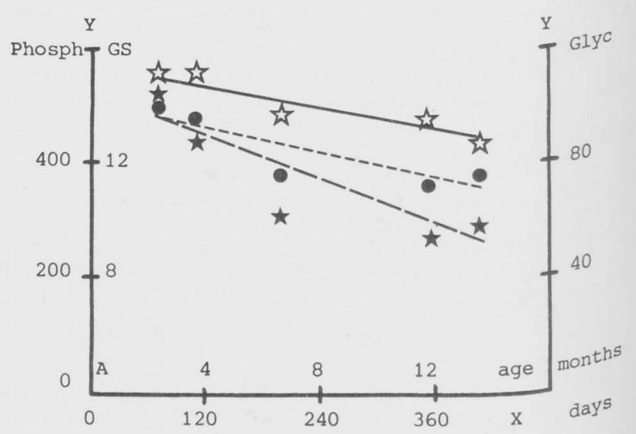


Figure 2 : EVOLUTION OF THE METABOLIC TYPE OF SOME LAMB MUSCLES DURING GROWTH BETWEEN 2 TO 13 MONTHS

Letters indicate muscle (see figure 1). Numbers indicate age (in months).

For simplicity only results from 2, 6 and 13 month old lambs are shown.





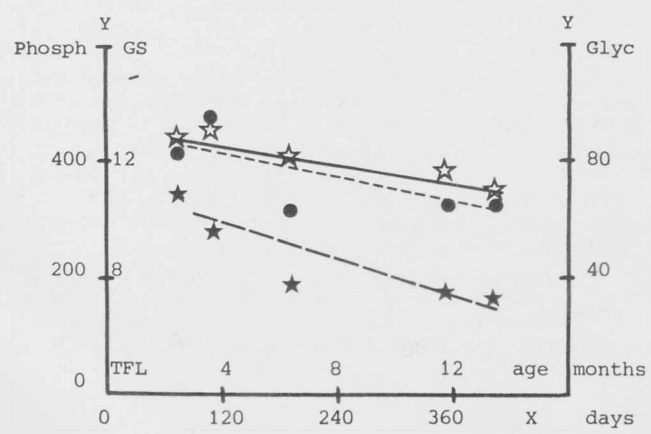
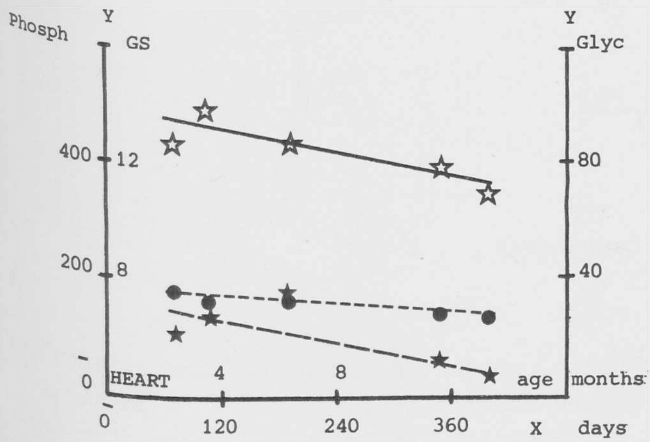


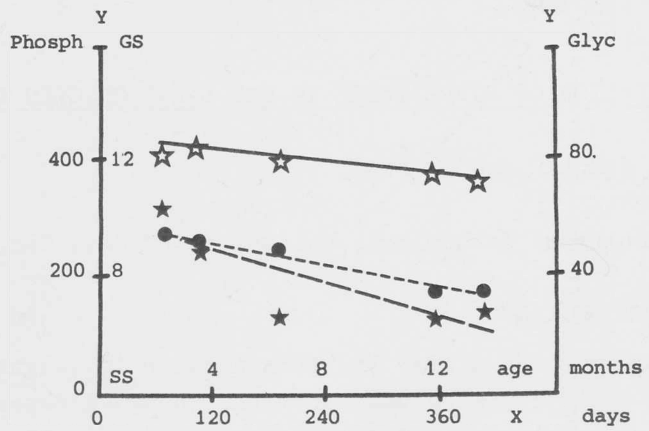
Figure 4 : EVOLUTION OF PHOSPHORYLASE, GLYCOGEN SYNTHETASE AND GLYCOGEN IN LD, A AND TFL MUSCLES OF LAMBS DURING GROWTH

★—— Glycogen synthetase (GS) (UI/g muscle protein)

●---- Phosphorylase (phosph) (UI/g muscle protein)

☆—— Glycogen (glyc) (μM/g muscle tissue)





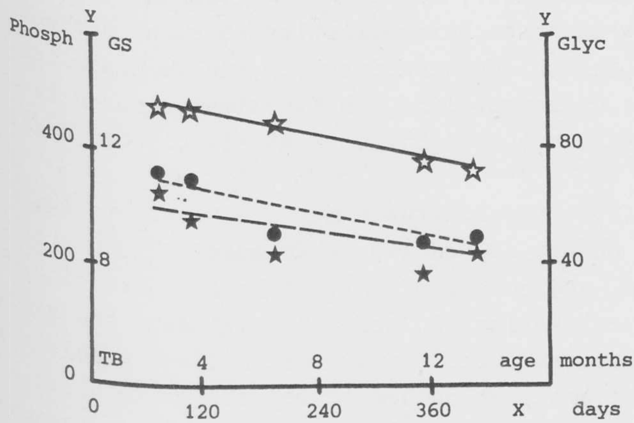


Fig. 3 : EVOLUTION OF PHOSPHORYLASE, GLYCOGEN SYNTHETASE AND GLYCOGEN IN HEART, SS AND TB MUSCLES OF LAMBS DURING GROWTH

★— — Glycogen synthetase (GS) (UI/g muscle protein)

●- - - - Phosphorylase (phosph) (UI/g muscle protein)

☆—— Glycogen (glyc) (μM/g muscle tissue)

Predominant metabolic type	Fast white		Fast red		Slow red	
muscle	TFL	ST	LD	A	TB	SS
Glycogen synthetase UI/g	1.9 ± 0.2	2.6 ± 0.2	2.9 ± 0.2	2.7 ± 0.1	2.2 ± 0.2	1.7 ± 0.1
Phosphorylase UI/g	62 ± 4	69 ± 4	77 ± 5	74 ± 4	51 ± 4	34 ± 2
Glycogen μM glucose/g	77 ± 11	74 ± 13	91 ± 11	101 ± 10	77 ± 9	76 ± 9

Table 1 : GLYCOGEN SYNTHETASE AND PHOSPHORYLASE ACTIVITIES, AND GLYCOGEN LEVEL IN VARIOUS LAMB MUSCLES (mean ± s.e.m. , n = 8)

All values are expressed on a fresh muscle weight basis

Muscles	Glycogen synthetase	Phosphorylase	Glycogen
Heart	$Y = 7.6 - 69.10^{-4} X$ xx	$Y = 195 - 0.14 X$	$Y = 99 - 66.10^{-3} X$
SS	$Y = 10.0 - 95.10^{-4} X$ xx	$Y = 255 - 0.27 X$	$Y = 88 - 38.10^{-3} X$
TB	$Y = 10.6 - 58.10^{-4} X$	$Y = 379 - 0.33 X$	$Y = 102 - 71.10^{-3} X$ xx
LD	$Y = 14.3 - 124.10^{-4} X$ x	$Y = 500 - 0.25 X$	$Y = 114 - 50.10^{-3} X$
A	$Y = 14.5 - 132.10^{-4} X$ xx	$Y = 501 - 0.35 X$ xx	$Y = 114 - 62.10^{-3} X$ xx
TFL	$Y = 11.2 - 92.10^{-4} X$ xx	$Y = 456 - 0.34 X$ xx	$Y = 92 - 60.10^{-3} X$ xx

Table 2 : REGRESSION OF ENZYME ACTIVITIES AND GLYCOGEN LEVEL ON AGE (n = 25)

Y : enzymes activities = UI/g muscle protein ; glycogen = μM glucose/fresh tissue ;
X : age = days

xx = significant at the $P < 0.01$ level ; all the other are significant at the $P < 0.05$ level