Residual glycogen at high ultimate pH in horse muscle

Except when starvation, or exercise immediately pre-slaughter, has seriously diminished glycogen reserves[1,2,3], the conversion of glycogen to lactic acid in mammalian muscle proceeds until a pH is reached when the enzymes effecting the breakdown become inactivated. In rabbit psoas muscle, this pH is about 5.3–5.4[1,4]: glycogen is generally considered to be absent from muscles having an ultimate[5] pH (*i.e.* the lowest pH finally attained) above this value, although BATE-SMITH[6] pointed out that about 20 mg/% of residual glycogen could be found in the leg muscles of beef at an ultimate pH of 5.7, and BENDALL[7] has recently found residual glycogen in various rabbit muscles, although not more than 20 mg/% above an ultimate pH of 5.8.

During an investigation on horse muscles[8], it was found that the ultimate pH of psoas and diaphragm was significantly higher than that of L.dorsi. Glycogen determinations were not carried out, however, and these findings were attributed to the absence of glycogen in the former two muscles, although it was noted that this did not accord with the reputedly high glycogen content of horse flesh.

Data on the residual glycogen in horse L.dorsi, psoas, diaphragm and heart have now been obtained. Together with data on initial pH, initial glycogen (*i.e.* at a standard time of 1 hour post-mortem) and ultimate pH they are given in Table I, and represent the mean (and S.E. of mean) of samples from six animals. Glycogen determinations were carried out by the method of GOOD, KRAMER AND SOMOGYI[9], pH was measured by the glass electrode, 2 g muscle being homogenized in 0.01 *M* sodium iodoacetate for this purpose.

In order to compare the findings with those of previous experiments, the determinations were made on muscle samples after they had been exposed to moist nitrogen for 5 hours at 37° C, followed by 17 hours at 0° C.

TABLE I

INITIAL AND ULTIMATE pH, AND INITIAL AND RESIDUAL GLYCOGEN, IN VARIOUS HORSE MUSCLES

Muscle	*Initial pH**	*Ultimate pH*	*Initial glycogen mg/100 g**	*Residual glycogen mg/100 g*	*Δ Glycogen / Δ pH*
Heart	6.00 ± 0.03	5.81 ± 0.05	584 ± 164	276 ± 101	1021
Psoas	6.77 ± 0.04	5.85 ± 0.06	1229 ± 190	666 ± 143	678
Diaphragm	6.97 ± 0.05	5.91 ± 0.04	1715 ± 142	1109 ± 116	572
L. Dorsi	6.87 ± 0.02	5.56 ± 0.03	2249 ± 210	1411 ± 228	640

* At 1 hour post-mortem.

It will be noted that the high ultimate pH of horse psoas and diaphragm (together with heart), previously attributed to the absence of glycogen[8], cannot, in fact, be due to this. Apparently the pH at which breakdown of glycogen to lactic acid ceases is characteristic for each muscle. This could be explained by postulating that, in each, the enzyme systems effecting the breakdown are of different constitution. Alternatively, it is feasible that more than one type of glycogen exists, characteristically different proportions of the forms being present in various muscles. These types might be distinguished either in their susceptibility, or in their accessibility, to attack by glycolytic enzymes under post-mortem conditions. There is some evidence that glycogen stores are not physiologically homogeneous in beef[10] and rat[11,12] muscles.

The table also indicates that the quantities of residual glycogen are characteristic of the muscles studied, being lowest in heart and highest in L.dorsi. From this finding, and the data on "initial" glycogen (*i.e.* at 1 hour post-mortem) it may be inferred that, *in vivo*, this order of difference also obtains, and reflects the degree of anaerobiosis associated with the normal function of the muscle.

The considerable disappearance of glycogen between pH 6.0 and 5.8 in the heart might suggest that, if lactic acid is being produced, this muscle has a much higher buffering capacity than the three skeletal muscles studied: in fact, it has less. The breakdown of the glycogen must therefore be attributed to the operation of some mechanism other than its conversion to lactic acid by anaerobic glycolysis. It has been shown that α-amylase and maltase are present in rabbit[13,14] and horse muscles[15] and produce dextrins, maltose and glucose by breakdown of glycogen. Such enzymes may be especially active in heart muscle.

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Uridine diphosphate acetylglucosamine phosphate and uridine diphospate acetylgalactosamine sulfate

In the past few years a number of compounds containing uridine pyrophosphate linked to a sugar have been isolated from various sources. Three compounds of this type which contain a partially identified N-acetylamino sugar were isolated by PARK from penicillin-inhibited *Staphylococcus aureus*[1], and compounds of this type accumulate in penicillin-inhibited *Lactobacillus helveticus* 335 also[2]. In addition, uridine pyrophosphate N-acetylglucosamine has been isolated from yeast[3], and presumptively identified in liver[4,5]. The purpose of the present communication is to report the identification of two additional compounds, isolated from hen's oviduct, which contain uridine pyrophosphate and an N-acetylamino sugar.

Large amounts of N-acetylamino sugar esters were detected in several animal tissues using a modification of the MORGAN AND ELSON reaction[6]. A hot water extract prepared from one pound of oviduct from laying hens (which contained especially large amounts of these esters, about 0.5 μM/g) was brought to pH 9 and placed on a column of Dowex-1 Cl (2% cross-linked), 5 cm^2 × 15 cm. The column was eluted by gradient elution employing mixtures of NaCl and HCl*. Three peaks in the elution diagram (Fig. 1) which contained both 260 mμ absorption and N-acetylamino sugar were further purified by anion exchange chromatography under conditions different from the original elution and by paper chromatography. Analytical data for these three compounds (UDPA$_1$, UDPA$_2$, and UDPA$_4$) are presented in Table I. Hydrolysis of each of the compounds in 0.01 *N* and 1 *N* HCl yielded uridine diphosphate (UDP) and uridine-5′ phosphate (UMP) respectively, identified by paper chromatography and by chemical and enzymic analysis of the eluted spots**. The compounds therefore differ only in the nature of their N-acetylamino sugar fragments.

UDPA$_1$ has the same location on the chromatogram and the same R_F in ethanol-ammonium acetate as yeast UDP N-acetylglucosamine[3], and its sugar fragment has the same R_F in ethyl acetate-pyridine-

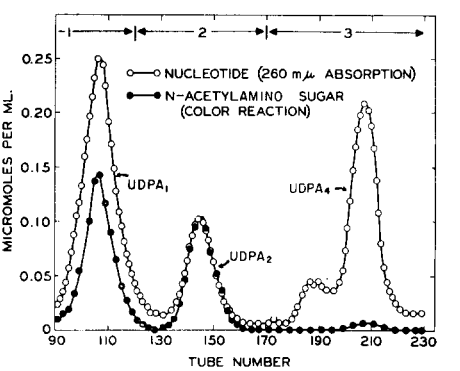

Fig. 1. Ion exchange chromatogram of hen's oviduct extract developed by gradient elution. In the portion of the chromatogram reproduced the gradients were: 1. 0.05 to 0.1 *M* NaCl in 0.01 *N* HCl, 2. 0.1 *M* to 0.25 *M* NaCl in 0.01 *N* HCl, 3. 0.01 *N* to 0.3 *N* HCl. The concentration of nucleotide was estimated approximately using the molar extinction coefficient for uridine, and N-acetylamino sugar was estimated using N-acetylglucosamine as the standard.

* The procedure used for the separation of tissue nucleotides, based on COHN's studies (*J. Cell. Comp. Physiol.*, 38 (Suppl. 1), 21 (1950)) of ion exchange chromatography of nucleotides, is very similar to the procedure employed by HURLBERT *et al.*[4]. The procedure differs in that 2% rather than 10% cross-linked resin has been used, and chloride rather than formate was the eluting anion.

** A third distinctly separate uridine nucleotide containing an N-acetylamino sugar (UDPA$_3$) was present between UDPA$_2$ and UDPA$_4$ on two occasions. Only small amounts of this compound were obtained, sufficient for identification of its hydrolysis products as UDP and UMP.