

incubation was slightly retarded by insulin. The magnitude of this insulin effect in adipose tissue is of the same order as the corresponding effect in frog muscle(5). In the latter tissue, insulin appears to increase [K] by increasing K influx without affecting K efflux (D. R. H. Gourley, unpublished experiments), but K fluxes in adipose tissue have not yet been studied. Although the insulin effect on adipose tissue [K] can occur in the absence of added glucose, glucose itself retards the loss of K during incubation. However, in the presence of glucose, the effect of insulin on adipose tissue [K] is more than 3 times greater than it is in the absence of glucose.

The effect of insulin on adipose tissue [Na] is less clear. Unfortunately, the extracellular space of the adipose tissue was not measured in these experiments but it is possible that a considerable fraction of the tissue Na is extracellular. Small changes in intracellular Na would therefore be more difficult to detect. The significance of the reduction in adipose tissue water by insulin in the presence of glucose is unknown but this response of adipose tissue to insulin seems to be unique.

Beigelman and Hollander(6,7) have reported that insulin, with or without added glucose, increases the resting potential of epididymal adipose tissue from rats weighing not more than 270 g. Without knowing the intracellular concentrations of Na and K, rigid comparisons with their data are not possible, but the net increase in adipose tissue [K] observed in the present experiments in the

presence of insulin is consistent with an insulin-induced increase in the membrane potential of individual cells. Nevertheless, these two effects may not be related causally because there is no evidence that glucose, which also increases the [K], has any effect on the resting membrane potential of adipose tissue.

Summary. In rats weighing 97-171 g, there is a significant negative correlation between the [Na] and [K] per g of water in freshly dissected epididymal adipose tissue and total body weight. During *in vitro* incubation in Krebs-Ringer's solution at 37°C, epididymal adipose tissue loses K and probably gains water and Na. After incubation, insulin-treated adipose tissue contains slightly more K and less Na than the corresponding control tissue. Glucose alone also increases the [K] of incubated adipose tissue. In the presence of glucose, the effect of insulin on adipose tissue K is considerably enhanced and the water content of the insulin-treated adipose tissue is significantly lower.

1. Forbes, G. B., Lewis, A. M., *J. Clin. Invest.*, 1956, v35, 596.
2. Thomas, L. W., *Quart. J. Exp. Physiol.*, 1962, v47, 179.
3. Martin, H. E., Tranquada, R. E., Beigelman, P. M., Jones, R., *Metabolism*, 1962, v11, 993.
4. Hausberger, F. X., Gujot, O., *Arch. Exp. Path. Pharm.*, 1937, v187, 647.
5. Manery, J. F., Gourley, D. R. H., Fisher, K. C., *Can. J. Biochem. Physiol.*, 1956, v34, 893.
6. Beigelman, P. M., Hollander, P. B., *Proc. Soc. Exp. Biol. and Med.*, 1962, v110, 590.
7. ———, *Diabetes*, 1963, v12, 262.

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Muscle Protein Extractability as Influenced by Conditions of Post-Mortem Glycolysis.* (29048)

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Muscle protein extractability at the time of death has been used as an indicator of *in vivo* protein composition(1). Mirsky(2) suggested that a large proportion of muscle

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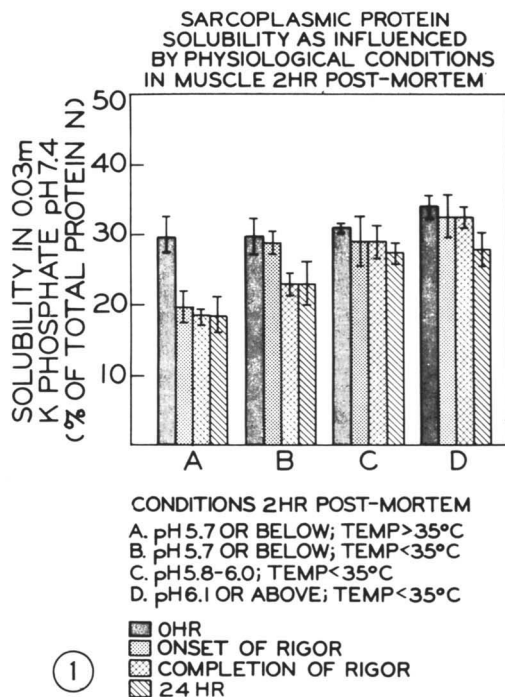


FIG. 1. Sarcoplasmic protein solubility as influenced by physiological conditions in muscle 2-hr post-mortem.

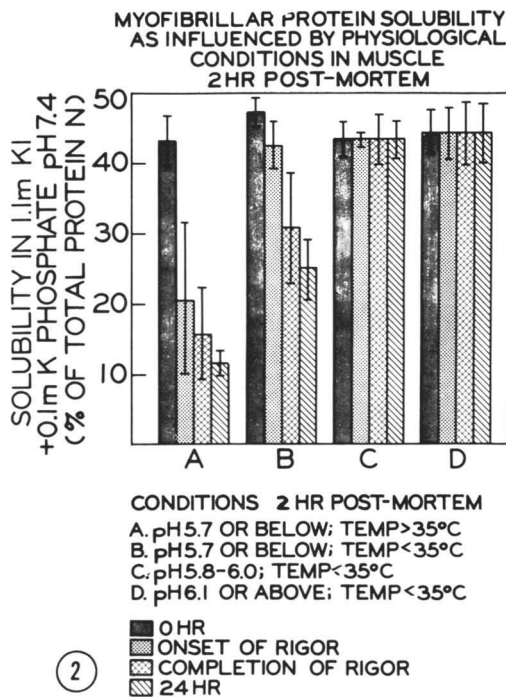


FIG. 2. Myofibrillar protein solubility as influenced by physiological conditions in muscle 2-hr post-mortem.

myosin becomes insoluble as rigor mortis develops. Bendall and Wismer-Pedersen(3) have stated that loss of myofibrillar protein solubility under conditions of rapid post-mortem glycolysis was the result of precipitation of denatured sarcoplasmic proteins on the fibrillar surface thereby markedly decreasing the extractability of muscle proteins. The present study was initiated to investigate the changes in the extractability of sarcoplasmic and myofibrillar proteins under various conditions of rigor mortis and glycolysis.

Porcine *longissimus dorsi* samples were frozen by immersion in a dry ice acetone bath immediately after death, at onset and completion of rigor mortis(4), and 24-hr. post-mortem. These samples were extracted according to the method of Helander(5) to determine solubility of sarcoplasmic and myofibrillar proteins. Results were grouped according to the conditions of temperature and pH in the muscle at 2 hours post-mortem. Fig. 1 shows that although sarcoplasmic pro-

tein solubility at 24 hours post-mortem was decreased from the initial solubility in all groups, it had decreased approximately 30-35% in Group A (> 35°) at the onset of rigor mortis and only 5-10% in Groups B, C and D (< 35°) at the same period. This suggested that under the pH conditions encountered in the muscle at 2 hours post-mortem the sarcoplasmic protein solubility remained high as long as the muscle temperature was below 35°C at 2 hours post-mortem. However, at the completion of rigor in Group B (< 35°; 5.7 pH) the sarcoplasmic solubility had significantly decreased.

Fig. 2 indicates that there were no changes in myofibrillar solubility at various post-mortem periods as long as the pH was medium to high (> 5.8; Groups C and D) and temperature low (< 35°) at 2 hours post-mortem. Under conditions of low pH (< pH 5.7) and low temperature (< 35°; Group B) at 2 hours post-mortem, the myofibrillar protein solubility also remained relatively high at

onset of rigor but decreased by 50% at 24 hours post-mortem. However, under conditions of low pH with temperatures above 35°C at 2 hours post-mortem (Group A) the myofibrillar protein solubility decreased by 56% at onset of rigor and by 74% at the 24-hour period. These data show that the solubility of both sarcoplasmic and myofibrillar proteins is markedly altered by post-mortem conditions of pH and temperature in the muscle. To obtain muscle protein extractability consistent with *in vivo* composition, it is essential to freeze the muscle samples immediately after death in a cryogenic liquid.

Loss of protein solubility is closely associated with rate of glycolysis and fluid retaining properties of the post-rigor muscle.

1. Dickerson, J. W. T., Widdowson, Elsie M., *Biochem. J.*, 1960, v74, 247.
2. Mirsky, A. E., *J. Gen. Physiol.*, 1936, v19, 571.
3. Bendall, J. R., Wismer-Pedersen, J., *J. Food Sci.*, 1962, v27, 144.
4. Briskey, E. J., Sayre, R. N., Cassens, R. G., *ibid.*, 1962, v27, 560.
5. Helander, Einar, *Acta Physiol. Scand.*, 1957, v41, 141.

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Lysozyme Activity in Radiation Chimeras. (29049)

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It was shown(1) that a marked increase occurred in lysozyme activity in the kidneys of irradiated mice treated with homologous bone marrow. This investigation was repeated, and lysozyme activity in different organs was determined.

Our interest in lysozyme stems from the problem of secondary disease or graft-against-host reaction in homologous bone marrow chimeras. In this immunologic disorder there appears to be a "metabolic starvation" that follows the immune reaction(2-4). The metabolic problem in secondary disease may be the important feature in causing death after treatment with foreign homologous bone marrow.

Even though the biological significance of lysozyme is unknown, it seems to be increased in mammals during immunological and other defense reactions.

A study of the striking changes in lysozyme in homologous bone marrow chimeras might contribute either to an understanding of the

biochemical role of lysozyme in tissues or to the mechanism of metabolic alterations during the foreign bone marrow reaction.

Materials and methods. Animals. Approximately 1000 male mice, 12 to 20 weeks old, were used in these experiments. Hybrid animals (101/Cum ♀ × C3H/Anf Cum ♂)F₁ were used as recipients or donors of isologous bone marrow, and (C57BL/6 Cum ♀ × DBA/2 Cum ♂)F₁ as donors of homologous bone marrow. They are referred to hereafter as IC3F₁ and B6D2F₁. These animals were divided into 4 groups: Group I were normal control mice, Group II were mice given 950 r total-body irradiation, Group III were mice irradiated (same dose) but given 40 × 10⁶ isologous bone marrow cells (IBM), and Group IV were mice irradiated (950 r) and given 40 × 10⁶ homologous bone marrow cells (HBM). The cells were given intravenously by tail vein within 5 hours after irradiation. They were kept 10 per cage and allowed free access to food and water.

The conditions of irradiation and technique for preparing bone marrow suspensions were the same as described previously(1).

Procurement of tissues. The animals were

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