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Insulin stimulation of [^{14}C]leucine incorporation into protein

It has been suggested that the primary effect of insulin is exerted at the extracellular–intracellular barrier of the cell, *i.e.*, the plasma membrane. In this way insulin initiates a sequence of intermolecular rearrangements which accounts for its effects on metabolism[1,2]. In regard to the incorporation of ^{14}C-labelled amino acids into protein, MANCHESTER[3] found that when isolated diaphragm was cut into successively smaller fragments prior to incubation *in vitro*, the influence of insulin progressively diminished. Furthermore, the repeated inability to demonstrate either a clear-cut or consistent effect of insulin added *in vitro* to a subcellular preparation is well known[4,5]. In this report we will present data consonant with the hypothesis that insulin requires an intact cell membrane in order to stimulate [^{14}C]leucine incorporation into protein and that this enhancement may be related to regulation of amino acid transport across the cell membrane.

Materials and methods were the same as described in earlier studies[6,7]. Additional details are given in the text and legends.

In Fig. 1 are shown the results of experiments wherein tissues obtained from untreated animals are incubated with and without insulin. It can be seen that stripping the tissue into halves optimizes both baseline incorporation of [^{14}C]leucine and insulin stimulation of this process. Stripping into quarters leads to reduction in both baseline and insulin-stimulated incorporation of [^{14}C]leucine. Stripping into eighths produces no further reduction in baseline incorporation, but a further reduction of insulin's effect occurs. The maintenance of baseline incorporation in going from quarters to eighths implies that the synthetic machinery remains relatively unharmed, while the concomitant vanishing of insulin's effect implies that some other essential cellular component, perhaps cell membrane, has been damaged.

It can be seen in Table I that tissues taken from rats which received insulin intraperitoneally incorporate more [^{14}C]leucine into 0.20 ionic strength extractable protein than controls. Here insulin was given *in vivo*, and the muscles were subsequently incubated *in vitro*. It should be noted in the case of stripped halves that both incorporation of [^{14}C]leucine into protein and insulin stimulation of this process are approximately the same whether insulin was first given *in vivo* (1 h before tissue sample preparation) 183 *vs.* 124 counts/min per mg (Table I), or whether it was added directly to the incubating flask 181 *vs.* 131 counts/min per mg (Fig. 1). Apart from the route of introducing insulin, both incubations were identical and of 1-h duration. This suggests that the observed insulin effect is "relatively primary", *e.g.*, at the cell

membrane and need not necessarily depend on the results of a time-consuming chain of events. The insulin effect seen by WOOL[8] at 5 min in rat diaphragm would further substantiate this suggestion. HANKING AND ROBERTS[9] found that when they prepared ribosomes and pH-5 enzymes from liver slices that had been incubated in the presence

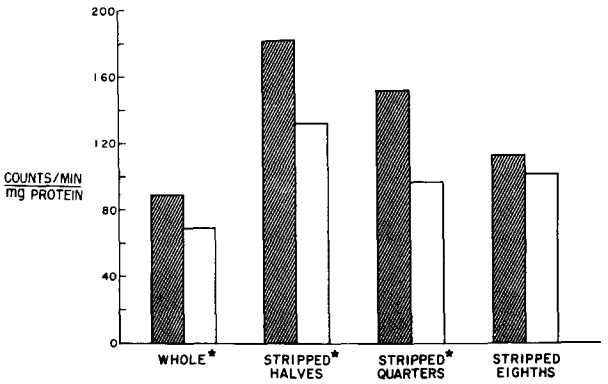

Fig. 1. Effect of muscle stripping on incorporation of [^{14}C]leucine into protein. Caudofemoralis muscles were excised from untreated animals fasted overnight and were incubated as whole preparations or stripped longitudinally into halves, quarters, or eighths. Incubation was carried out in 5 ml of Krebs–Ringer phosphate buffer with half Ca^{2+} concentration at pH 7.2 in the presence of 18 amino acids at concentrations approximating normal intracellular levels in rat thigh muscle[7]. Insulin was present in half the flasks at 0.1 unit/ml. After 1 h of incubation at 37°, tissues were homogenized at ionic strength 0.20. Aliquots of the homogenate supernatant were prepared for protein determination and liquid scintillation counting as described[6,7]. Cross hatched areas, insulin treated; open bars, controls.

* $P < 0.05$.

TABLE I

EFFECT *in vitro* OF SYSTEMICALLY ADMINISTERED INSULIN

4 normal rats fasted overnight were injected intraperitoneally with 0.5 unit of insulin while 4 controls received saline. Rats were sacrificed 1 h later, caudofemoralis muscles were stripped into halves and incubated. Remainder of procedure as in Fig. 1.

Counts/min per mg protein (± S.E.)		*Absolute stimulation*	*% Stimulation*
*Insulin**	*Control*		
183 ± 13	124 ± 13	59	48

* $P < 0.001$.

of increasing concentrations of amino acids, there was an increase in both the ability of these ribosomes to incorporate amino acids and of the pH-5 enzymes to support amino acid incorporation into control ribosomes. EAGLE[10] observed that within a range of intracellular concentrations for various amino acids in cultured mammalian cells, rates of protein synthesis and cell growth varied directly with the amino acid concentration. The intracellular amino acid concentration may influence the critical ratio between acylated and de-acylated transfer RNA which has been invoked as a translational control mechanism for protein synthesis[11–16]. The role of insulin in promoting protein synthesis may be envisaged as regulating the flow of extracellular

amino acids to acylated transfer RNA by augmenting transport either by a direct effect on the cell membrane or *via* an intramembrane-generated intermediary.

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