Other materials used on occasion as inhibitors of protein synthesis include amino-acid and nucleotide analogues. Neither azauridine (1 mmolar), 5-fluorouracil (10 μ molar), DL-norleucine (20 mmolar) nor DL-ethionine (20 mmolar) have any appreciable inhibitory influence on incorporation by diaphragm even during incubation periods as long as 6 h. It could be argued that the materials do not readily penetrate the tissue—but the uptake of amino-acids is well established, the incorporation of nucleotides readily observable and the rapidity with which molecules as large as puromycin, actinomycin and cycloheximide exert their influence is impressive. Uptake of chloramphenicol by reticulocytes has recently been studied²³.

Table 3. EFFECT OF INSULIN IN THE PRESENCE OF CYCLOHEXIMIDE ON THE ACCUMULATION AND INCORPORATION INTO PROTEIN OF ¹⁴C-GLYCINE BY ISOLATED RAT DIAPHRAGM

Insulin added	¹⁴ C-glycine accumulated (Ratio c.p.m./ml. tissue water c.p.m./ml. medium)	Radioactivity incorporated into protein (c.p.m./mg protein)
—	2.04 $\pm$ 0.18	16.5 $\pm$ 0.70
+	2.95 $\pm$ 0.21 $P = 0.01$	22.3 $\pm$ 1.10 $P = 0.001$

Each figure is the mean $\pm$ standard error of mean of six observations. The tissue was pre-incubated for 30 min in the presence of 2 μ g/ml. of cycloheximide before addition of the ¹⁴C-glycine (0.2 μ l./ml.).

Table 4. EFFECT OF ADMINISTRATION OF CYCLOHEXIMIDE *in vivo* ON THE CAPACITY OF THE SUBSEQUENTLY ISOLATED DIAPHRAGM MUSCLE TO INCORPORATE ¹⁴C-GLYCINE INTO PROTEIN AND TO RESPOND TO INSULIN

Animals injected with	Isolated diaphragm with insulin	Uptake of glucose (mg/g wet weight/h)	Radioactivity incorporated into protein (c.p.m./mg of protein)
Saline	—	3.58 $\pm$ 0.25	145 $\pm$ 22
Saline	+	6.38 $\pm$ 0.20 $P < 0.001$	243 $\pm$ 16 $P < 0.01$
Cycloheximide	—	2.89 $\pm$ 0.31	21.7 $\pm$ 0.92
Cycloheximide	+	6.55 $\pm$ 0.17 $P < 0.001$	30.4 $\pm$ 2.90 $P < 0.02$

Each figure is the mean $\pm$ standard error of mean of six observations. The cycloheximide (10 mg/150 g of rat²⁹) was injected intraperitoneally (in saline) 90 min before removal of the diaphragm.

Extensive incubation with puromycin lowers the capacity of muscle to take up amino-acids²⁶, although the effect is not seen in shorter incubations²⁶. This suggested that the membrane carrier for amino-acids may be a protein with a rapid rate of turnover. Wool and Cavicchi²⁷ have also provided evidence that puromycin and cycloheximide interfere with the response of a muscle ribosomal system to insulin, and they suggest, by analogy with similar findings with adrenocorticotrophic hormone²⁸, that a primary action of insulin is to promote formation of a labile protein which is responsible for increasing the activity of the polysomes. I have confirmed the findings²⁵ of the inhibitory effect of puromycin and cycloheximide on accumulation of aminoisobutyrate (Table 2). But an effect of insulin to promote aminoisobutyrate accumulation remains observable, albeit on a smaller absolute scale, and promotion of glucose uptake is also seen. A normal percentage increase of incorporation by insulin is observable (Table 3) although it is reduced by pre-incubation with cycloheximide. When cycloheximide is administered *in vivo* before removal of the diaphragm, the tissue again responds to insulin both with respect to glucose uptake and amino-acid incorporation (Table 4). These observations do not support the concept that the primary actions of insulin on muscle, whatever they are, are dependent on proteins with a rapid rate of turnover and renewal. Because cycloheximide is thought to bind to ribosomes, a more mundane interpretation of the results of Wool and Cavicchi²⁷ and possibly those of Garren *et al.*²⁸ would be that the ribosomal systems isolated after treatment with cycloheximide remain contaminated with the inhibitor and, despite some apparent incorporating capacity, are effectively dead. In these conditions hormonal response is unlikely to be observed. The question remains why cycloheximide *in vivo* was not found totally to inhibit incorporation by diaphragm. This is probably a technical question of dosage

as can be shown with puromycin¹⁷. A more important question is why ribosomal systems from animals treated with inhibitors exhibit incorporating capacity at all (for example, refs. 29, 30). Without a clearer understanding of the full enzymology of protein synthesis this is difficult to answer.

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Growth Hormone and Carbohydrate Metabolism *in vitro*

IN the recent communication under this title, Bolodia and Young¹ say that "... no satisfactorily reproducible effect of growth hormone *in vitro* on carbohydrate metabolism has been described". No grounds are given for rejecting the comprehensive evidence of Ottaway and Bulbrook² for effects on the glucose uptake of rat diaphragm which are practically identical with those now described by Bolodia and Young, nor for the evidence of Bronk and Fisher³ that very small concentrations of bovine growth hormone produce anti-insulin effects on the perfused rat heart. Bronk and Fisher showed that growth hormone reduced glucose uptake and permeability to galactose in the absence of insulin as well as in its presence. It follows that growth hormone produces an effect which offsets the action of insulin but is not acting as an anti-insulin.

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