1951) showed that fructose metabolism was unaffected by the diabetic condition whereas that of glucose was altered. Fructose has been shown to lower ATP concentrations and those of cyclic AMP, increased previously by glucagon, and decrease phosphorylase 'a' in the liver of normal mice (van den Berghe et al., 1973). These effects of fructose could partly account for the results of our experiments, since it is known that diabetic animals have increased hepatic cyclic AMP concentrations (Das, 1973) and thus possibly increased phosphorylase activity, as well as having a lower percentage of glycogen synthetase in the 'a' form (Steiner et al., 1961). However, as the addition of fructose, with glucose, to the perfusion medium did not produce significant glycogen accumulation, it appears that the hexoses must be acting together with other factors during pretreatment of diabetic rats, to produce the eventual changes (during perfusion) in the enzymes concerned with glycogen metabolism.

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Insulin-Induced Adenosine 3':5'-Cyclic Monophosphate-Independent Phosphorylation of a Fat-Cell Protein: Effect of Starving and Re-feeding
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The initial steps in the cascade of reactions produced by insulin are believed to be hormone recognition and interaction with a specific receptor in the cell membrane (Krahl, 1961; Cuatrecasas, 1972). In order for events at the hormone-cell-membrane-receptor level to produce diverse effects on intracellular metabolism, a transducing system must convey both the qualitative and quantitative nature of the membrane interaction to the appropriate effector system. Protein kinases are particularly important in these information-transfer systems (Greengard & Kuo, 1970), and cyclic adenosine 3':5'-monophosphate specifically activates several of these enzymes (Walsh et al., 1968; Rubin et al., 1972). However, if the adenylate cyclase–hormone effector system is used as the model for insulin action, it appears that only some of the effects of insulin on lipid, carbohydrate, protein, water and electrolyte metabolism are explained by insulin's ability to modulate the concentrations or effects of cyclic AMP. Although insulin may affect both adenylate cyclase (Illiano & Cuatrecasas, 1972) and phosphodiesterase activities (Manganiello & Vaughan, 1973), there is a dissociation between at least some of insulin's
actions and the intracellular concentrations and effects of cyclic AMP (Khoo et al., 1973). These observations suggest that an insulin-dependent protein kinase system, separate from the cyclic AMP-dependent protein kinase system, is a likely link between the insulin–membrane receptor complex and some biochemical responses to insulin.

Results of studies of fat cells in vitro (Benjamin & Singer, 1974a) are consistent with just such a mechanism of insulin action, i.e. a non-cyclic AMP-dependent action of insulin on protein phosphorylation. In either the absence or the presence of adenylate cyclase stimulation, physiological concentrations of insulin as low as 25 μ·i.u./ml specifically increased $[^{32}P]P_i$ incorporation into a high-molecular-weight fat-cell protein/polypeptide of approx. 140000 daltons (IPP 140). This insulin-stimulated phosphoprotein phosphorylation occurred within 15 min after the addition of hormone, and was unaffected by the absence of glucose. Further, turnover studies indicated that protein IPP 140 is dephosphorylated more rapidly than any other major fat-cell phosphoprotein (Benjamin & Singer, 1974b).

In addition, we have shown that adrenaline and dibutyryl cyclic AMP (Benjamin & Singer, 1974c) specifically increased the incorporation of $[^{32}P]P_i$ into a protein/polypeptide, designated adrenaline-phosphorylated protein, with a molecular weight of approx. 60000–65000 (EPP 60–65). Phosphorylation of protein EPP was found to be specifically antagonized by either Li+ or insulin. The present results are consistent with the suggestion that adrenaline action stimulates cyclic AMP-dependent protein kinase, whereas Li+ and insulin modulate this effect, either by inhibiting adenylate cyclase (Singer & Rotenberg, 1973; Illiano & Cuatrecasas, 1972), or by increasing phosphodiesterase activity (Manganiello & Vaughan, 1973).

Insulin-induced phosphorylation of fat-cell proteins was measured (Benjamin & Singer, 1974a) under conditions of prior stimulation of cyclic AMP-dependent protein kinase, and in fat-cells from starved and re-fed animals (Benjamin & Singer, 1974b). Protein phosphorylation was measured by incubating fat-cells (Rodbell, 1964) with $[^{32}P]P_i$ (50–100 μCi/ml) for short periods of time, with the addition of appropriate hormones. Fat-cells were collected after incubation, and the proteins were processed for analysis on sodium dodecyl sulphate–polyacrylamide slab gels by electrophoresis and high resolution radioautography (Benjamin & Singer, 1974a; Maizel, 1971). Fat-cells were prelabelled for 30 min with $[^{32}P]P_i$ and dibutyryl cyclic AMP to label the intracellular phosphate pool and to stimulate cyclic AMP-dependent protein kinase activity; Li+ was also present to inhibit adenylate cyclase. Unlabelled phosphate was then added to stop further labelling of the $P_i$ pool. Under these combined experimental conditions, insulin (50 μ·i.u./ml) markedly increased phosphorylation of protein IPP 140. These experiments indicate that insulin stimulates phosphorylation of protein IPP 140 even under conditions of simultaneous adenylate cyclase inhibition and cyclic AMP-dependent protein kinase stimulation, suggesting that the control of the biochemical pathways of phosphorylation of protein IPP 140 is independent of the cyclic AMP systems. These results also suggest that the proximate intracellular phosphate donor to protein IPP 140 is not $P_i$.

To determine whether these observations on the phosphoprotein phosphorylations of normal fat-cells from fed rats are related to normal biochemical events in vivo, fat-cells were obtained from rats maintained under different physiological conditions (starved and re-fed). If our previous observations on fat-cell phosphoprotein phosphorylation are related to normal physiology, then one would predict that when fat-cells from starved rats (associated with decreased insulin concentrations in vivo) are compared with those from re-fed rats (associated with intense fat-cell metabolic activity and increased insulin concentrations), phosphorylation of protein IPP 140 should be increased in cells prepared from re-fed rats. In four separate experiments with starved and re-fed animals, with cells assayed for phosphorylation of protein IPP 140 in vitro, we found a marked increase in phosphorylation of protein IPP 140 in the cells from re-fed rats compared with those from starved animals. In addition, cells from starved animals were able to respond to added insulin with an increase in phosphorylation of protein IPP 140, indicating that the cells from starved animals had hormonal competence.
These results support our hypotheses (a) that insulin specifically enhances the phosphorylation of protein IPP 140, (b) that this specific protein phosphorylation is not part of a cyclic AMP-dependent system, and (c) that this cyclic AMP-independent mechanism forms part of the normal intracellular circuitry of insulin action.

Illiano, G. & Cuatrecasas, P. (1972) Science 175, 906–908

High Intake of Vitamin C in Relation to Adenosine 3':5'-Cyclic Monophosphate and Guanosine 3':5'-Cyclic Monophosphate Concentrations and to Blood Sugar Concentrations

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An interrelationship between the activities of vitamin C, 3':5'-cyclic AMP and 3':5' cyclic GMP with respect to treatment of hyperglycaemia can be shown on the basis of the following. (1) Insulin lowers the concentration of glucose in blood. (2) High intake of vitamin C tends to result in lower blood-glucose concentrations. Several workers have shown that intravenous uptake of vitamin C (in the region of 0.3–1.2 g) results in significant lowering of the blood sugar curves in normal and in diabetic patients (e.g. Secher, 1942; Sylvest, 1942). Further, Pfleger & Scholl (1937) and Bartelheimer (1939) noted that high intake of ascorbic acid led to a decrease of the required insulin dose in several diabetic patients; and Dice & Daniel (1973) concluded that in one diabetic patient each gram of the vitamin C taken resulted in a decrease of the insulin dose required by 21 u. (3) Cyclic AMP and cyclic GMP activities influence, or are influenced, by insulin activity. (a) Cyclic AMP is known to enhance insulin release from the pancreatic islets (e.g. Sussman & Vaughan, 1967; Malaise et al., 1967). (b) Insulin affects adversely cyclic AMP concentrations (Senft et al., 1968). (c) Very high cyclic AMP concentrations are found in the pancreatic islets of rats (Turtle & Kipnis, 1967). (d) Insulin potentiates cyclic GMP in adipose and liver cells (Illiano et al., 1973). Cyclic AMP and cyclic GMP are mutually antagonistic (Goldberg et al., 1973). (4) Vitamin C enhances high concentrations of cyclic AMP and cyclic GMP. (a) The similarity in the stereochemistry of the anionic-ring sections of the two cyclic phosphates and of ascorbate indicate parallel reactivities with respect to substrate competition for the active sites in phosphodiesterase (Lewin, 1974c, 1975). (b) It has been experimentally established that ascorbate inhibits hydrolytic breakdown of cyclic AMP by phosphodiesterase (Moffat et al., 1972; Lewin, 1974c, 1975). (c) It has been experimentally established that multi-gram intake of ascorbic acid results in increased excretion of cyclic AMP in the urine (A. C. Owen & P. Moffat, personal communication). This has been confirmed (Lewin, 1975). The observation signifies increased serum cyclic AMP concentrations. (d) Enhanced