Table 1. *Effect of rabbit serum proteins on the incorporation in vitro of [1-¹⁴C]glycine into protein of Yoshida ascites-hepatoma cells from actinomycin D-treated and untreated rats*

The results are given as means $\pm$ S.E.M. of five experiments.

Rats	Incorporation (counts/min./mg. of protein)		
	Control	γ -Globulin	Albumin
Untreated	858 $\pm$ 115	3524 $\pm$ 608	2525 $\pm$ 671
Actinomycin D-treated	729 $\pm$ 58	983 $\pm$ 64	914 $\pm$ 23

both globulins and albumins from normal rabbit serum greatly stimulate the incorporation *in vitro* of radioactive glycine into protein of hepatoma cells ($P < 0.01$), globulins appearing to be more effective than albumins in producing the stimulation. The results also show that pretreatment of tumour-bearing rats with actinomycin D does not significantly affect the incorporation in the basal medium ($P > 0.3$), whereas it abolishes almost completely the stimulating effect of serum proteins ($P < 0.01$).

The study of the kinetics of the incorporation has shown that, in cells from both actinomycin D-treated rats and controls, the rate of incorporation proceeded as a linear function of the time from the beginning up to at least 1 hr., both in the presence and absence of serum globulins.

To conclude, the results presented show clearly that the stimulating effect of serum proteins on the incorporation of labelled glycine into protein of Yoshida ascites-hepatoma cells is abolished by pretreatment of tumour-bearing rats with a dose of actinomycin D that practically does not affect the basal level of incorporation. Thus, on the basis of what is known on the mechanism of action of actinomycin D (Collins, 1965; Reich, 1964), it appears reasonable to conclude that serum proteins affect the transcription reactions in some way.

Two main problems arise at this point. The first one is whether the stimulation of the incorporation brought about by serum proteins is due to the protein molecule itself or to the products of its degradation. That such an effect might be due to simple molecules such as amino acids was excluded by separate experiments showing that a mixture of amino acids reproducing the composition of normal rabbit serum γ -globulin (except tryptophan) (Porter, 1959) actually depressed the glycine incorporation, most probably because of the dilution of the marker. On the other hand, the possibility cannot be ruled out that the stimulation of protein synthesis by rabbit serum proteins is due to peptides arising from the degradation of these proteins. There are several examples of peptides, hormonal and otherwise, having a stimulatory effect on protein synthesis.

The second problem is how rabbit serum proteins, or peptides derived from their degradation, bring about the stimulation of protein synthesis. This problem is now under investigation in this Laboratory from several points of view, and it has been shown (G. Marsilii & V. Chiarugi, unpublished work) that the addition of normal rabbit serum globulins to hepatoma-cell suspensions is followed by an enhanced nuclear synthesis of rapidly labelled RNA.

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The Effects of Sugars on Incorporation of [³H]Leucine into Insulins

By D. G. PARRY and K. W. TAYLOR

Department of Medicine and Diabetic Department, King's College Hospital, London, S.E. 5

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Increasing the glucose content of the medium in which slices of ox pancreas are incubated greatly stimulates the incorporation of labelled leucine

into ox insulin (Taylor, Parry & Smith, 1964). This effect appears to be specific for insulin, and alterations in the sugar content of media in which

pancreas slices were incubated had no effect on the incorporation into acid-ethanol extracts of other pancreatic proteins.

These studies have now been extended to the effects of other sugars on the incorporation of leucine into insulin in slices of both ox pancreas and rabbit pancreas. It is shown below that increasing the concentrations of glucose and mannose in the medium increases incorporation into insulins, though this is not true for galactose. D-Mannoheptulose inhibits the effects of glucose on incorporation *in vitro*. Incubation of slices of pancreas and subsequent extractions after incubation were carried out as described in detail elsewhere (Mallory, Smith & Taylor, 1964; Taylor, Gardner, Parry & Jones, 1965), except that insulin was estimated by immunoassay. In general, fresh pancreas slices obtained from the slaughter-house for ox pancreas, or from freshly killed rabbits, were incubated with [^3H]leucine of high specific activity and the appropriate sugars in a bicarbonate-buffered medium for 3 hr. periods. At the end of this time, pancreas slices were dropped into acetone-solid CO_2 mixtures and the insulin was extracted by acid-ethanol.

Insulin extracted from the incubated slices was purified by immuno-precipitation and was further characterized by two-dimensional paper chromatography and electrophoresis in 20% (w/v) formic acid at pH 1.8 (Taylor & Smith, 1964; Taylor *et al.* 1965). Rabbit insulin, like ox insulin, may be purified by precipitation with antibody made against ox insulin. Radioactivity in insulins was determined on paper as described by Taylor *et al.* (1965). Insulin was estimated in acid-ethanol

extracts of the precipitate obtained with antibody by immunoassay, by the method of Hales & Randle (1963), by reference to ox insulin standards.

Reasons for accepting the general validity of techniques of this kind for studying biosynthesis *in vitro* have been presented elsewhere (Mallory *et al.* 1964; Humbel, 1963, 1965). With this technique the A-chain of both ox insulin and rabbit insulin may be shown to be labelled. Moreover, electron microscopy of such tissues suggests that, after incubation, pancreas slices remain normal in appearance (J. R. Williamson, P. E. Lacy & K. W. Taylor, unpublished work).

The effect of alterations in sugar content of the incubation medium on the incorporation of [^3H]leucine into rabbit insulin and ox insulin is shown in Table 1. Increasing the concentration of glucose substantially increases the rate of incorporation of leucine into insulins of both species. Mannose shows a similar effect to glucose in experiment with ox pancreas.

By contrast, incorporation of [^3H]leucine into insulin in the presence of galactose (250 mg./100 ml.) is much lower than when slices are incubated with a high concentration of other sugars. Mannoheptulose appeared to inhibit incorporation into rabbit insulin when used at a concentration of 250 mg./100 ml. in the presence of glucose. At this concentration it has been shown to suppress secretion of insulin from rabbit pancreas *in vitro* (Coore, Randle, Simon, Kraicer & Shelesnyak, 1963).

Though it has long been known that high concentrations of sugars can release insulin from pancreatic islets, it has been much less evident

Table 1. *Effect of sugars on the incorporation of [^3H]leucine into ox insulin and rabbit insulin in vitro*

Pancreas slices were incubated with DL-[4,5- $^3\text{H}_2$]leucine in bicarbonate buffer for 3 hr. with appropriate sugars. After incubation insulin was extracted by acidified ethanol and purified by immuno-precipitation. After chromatography and electrophoresis, insulin was counted on paper. Further details of methodology are given in text. The results are given as means $\pm$ s.e.m. of the numbers of observations in parentheses.

Species	Sugar in incubation medium	Radioactivity in insulin	
		Control value (counts/min./ $\mu\text{g.}$ of insulin with sugar at 250 mg./100 ml. in medium)	Percentage of control value with sugar at 25 mg./100 ml. in medium
Ox	Mannose	9.9	16.8 $\pm$ 3 (5)
	Glucose	6.0	27.0 $\pm$ 17 (3)
	Galactose	1.9	94.6 $\pm$ 43 (5)
Rabbit	Glucose	96.6	7.7 $\pm$ 4 (3)
			Percentage of control value with mannoheptulose at 250 mg./100 ml. in medium
	Glucose	99.0	61 $\pm$ 11 (5)

whether or not sugars possess any direct effect on insulin synthesis. From the results shown in Table 1, it is clear that both glucose and mannose can increase leucine incorporation into insulin. Both these sugars are known to provoke the secretion of insulin *in vitro* (Coore & Randle, 1964a) and *in vivo* (Karam, Grodsky & Forsham, 1965).

A further similarity between effects of sugars on synthesis and secretion is seen when mannoheptulose is incubated with pancreas slices. This sugar inhibits the incorporation of leucine in the presence of glucose in a way that is very similar to its known inhibition of insulin release *in vitro* (Coore *et al.* 1963).

It is suggested that both synthesis and secretion of insulin may be linked within the islet cell. If this is the case then linkage may take place at the stage of sugar phosphorylation. Both glucose and mannose are similarly phosphorylated by many tissues either by hexokinase or glucokinase. Moreover, D-mannoheptulose is known to inhibit phosphorylation of sugars in liver extracts (Coore & Randle, 1964b). It is not known how the further metabolism of glucose phosphates might influence insulin synthesis within β -cells. Synthesis of insulin might be peculiarly dependent on the provision of energy from oxidation of sugars, although in other tissues (such as muscle) incorporation of labelled amino acids into protein seems to be independent of glucose concentration (Manchester & Young, 1959). Alternatively, an increased oxidation of glucose through the pentose phosphate shunt might be related to increased ribosomal activity for insulin synthesis. The pentose phosphate shunt is known to be especially active in islet cells (Field, Johnson, Herring & Weinberg, 1960; Lazarow, 1963).

An effect of sugars in increasing the rate of insulin synthesis would seem to have physiological

importance in preventing an undue depletion of islet-cell insulin under conditions of maximal stimulation. It may explain why the total insulin content of rat pancreas is only moderately decreased even after many hours of infusion of glucose at a high concentration (Logothetopoulos, Kaneko, Wrenshall & Best, 1964).

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Hydrogen Ion/Oxygen, Hydrogen Ion/Calcium Ion and Calcium Ion/Oxygen Ratios during Aerobic Uptake of Calcium Ions in Liver Mitochondria

By C. ROSSI, A. AZZI and G. F. AZZONE

Unit 'G. Vernoni' for the Study of Physiopathology, Institute of General Pathology, University of Padua, Italy

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The H^+/Ca^{2+} and Ca^{2+}/O ratios during aerobic Ca^{2+} uptake have usually been measured under conditions where limited amounts of Ca^{2+} were

added to mitochondria incubated in the presence of an excess of substrates in a tris-HCl-buffered medium. Under these conditions a H^+/Ca^{2+} ratio