Alterations in Regulation of Insulin Biosynthesis in Pregnancy and Starvation Studied in Isolated Rat Islets of Langerhans

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1. Insulin biosynthesis in isolated rat islets of Langerhans was determined by the incorporation of [^3H]leucine into newly synthesized islet proteins. Anti-insulin serum covalently coupled to a solid phase (CNBr-activated Sepharose 4B) was used to separate the immunoreactive proinsulin and insulin from other islet proteins. This method was applied to a study of the regulation of insulin biosynthesis in isolated rat islets of Langerhans during pregnancy, and immediately after a period of food deprivation. 2. Islets isolated from pregnant rats showed an increased basal rate of synthesis compared with the non-pregnant controls. In addition, they showed a significant increase in biosynthesis of proinsulin and insulin in comparison with the normal islets over a range of glucose concentrations of 2–20 mM. 3. Addition of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine significantly increased the insulin-synthetic response of normal islets over the glucose range 5–20 mM, so that their glucose response approached that of islets from pregnant rats. 4. Normal female rats were injected with a long-acting progesterone derivative (hydroxyprogesterone hexanoate), to investigate the role of progesterone on the increased insulin biosynthesis observed in islets in pregnancy. There appeared to be no marked difference in insulin biosynthesis between the islets from the progesterone-injected and control rats in the presence of 2 mM- or 6 mM-glucose alone. However, in the presence of 4 mM- or 6 mM-glucose and 3-isobutyl-1-methylxanthine there was a significant increase in insulin biosynthesis in the progesterone-treated animals. 5. Total islet protein biosynthesis was determined by the incorporation of [^3H]leucine into trichloroacetic acid-precipitable islet proteins. Islets isolated from normal rats showed a 1.6-fold increase in incorporation over the glucose concentration range 2–20 mM, and this value remained unchanged during starvation; however, rates of incorporation were significantly raised in islets isolated from pregnant rats in the presence of 20 mM-glucose. 6. Islets from starved and fed control rats were incubated in the presence of increasing concentrations of glucose or glucose+3-isobutyl-1-methylxanthine. The islets isolated from the starved animals showed a diminished insulin-synthetic response to glucose as compared with the controls; this response was partially restored to normal values by elevation of cyclic AMP concentrations by using 3-isobutyl-1-methylxanthine. 7. It is suggested that the alterations in glucose-stimulated insulin biosynthesis observed in islets during pregnancy and after a period of starvation could be attributable, at least in part, to a long-term alteration of the cyclic AMP system, and in pregnancy to a direct or indirect effect of progesterone on β -cell function.

Rates of insulin biosynthesis and secretion are known to be regulated in the short term by many factors, and this short-term regulation has been extensively investigated (Steiner *et al.*, 1972; Lernmark *et al.*, 1976; Gerich *et al.*, 1976). It is now clear, however, that there is superimposed on this acute control a longer-term regulation of secretion which is sensitive to changes in physiological circumstances. This has been most extensively documented in starvation (Malaisse *et al.*, 1967; Grey *et al.*, 1970a), in which glucose-induced insulin secretion is

diminished, and in pregnancy (Malaisse *et al.*, 1969; Costrini & Kalkhoff, 1971; Green & Taylor, 1972), in which insulin-secretory responses to glucose are enhanced.

The purpose of the present study was to determine whether the alterations of the regulation of insulin secretion in pregnancy and starvation were paralleled by comparable changes in the long-term regulation of insulin biosynthesis. A brief preliminary report of some of these studies has been published (Bone & Taylor, 1976).

Experimental

Reagents

The Radiochemical Centre (Amersham, Bucks., U.K.) supplied L-[4,5-³H]leucine of specific radioactivity 53 Ci/mmol. CNBr-activated Sepharose 4B was from Pharmacia (G.B.) Ltd., London W.5, U.K. 3-Isobutyl-1-methylxanthine was obtained from Aldrich Chemical Co., Milwaukee, WI, U.S.A. Primolut Depot (progesterone; hydroxyprogesterone hexanoate) was from Schering Chemicals, Pharmaceutical Division, Burgess Hill, Sussex, U.K. Soluene 350 tissue solubilizer and dimethyl-POPOP [1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene] were obtained from Packard Instruments, Caversham, Berks, U.K., and PPO (2,5-diphenyloxazole) was from Koch-Light Laboratories, Colnbrook, Bucks., U.K.

Methods

Animals. Female Sprague-Dawley rats weighing 250–300 g were used. Pregnancy was achieved by natural timed matings; age-matched normal and 19-day-pregnant rats, which were allowed free access to both food and water, were used in the experiments. In some experiments, animals were starved but allowed unlimited water during the 48 h before death; in other experiments normal female rats were injected with 25 mg of progesterone (hydroxyprogesterone hexanoate), twice over a 10-day period.

Incubation of islets. Islets were isolated by the collagenase digestion of pancreas tissue as described by Howell & Taylor (1968). Groups of 15–20 islets were incubated in 0.5 ml of bicarbonate-buffered medium, pH 7.4 (Gey & Gey, 1936), previously gassed with O₂/CO₂ (19:1). The medium was supplemented with 1 mg of bovine serum albumin/ml and contained various concentrations of glucose alone or with the additions as indicated in the Results section, together with 20 μ Ci of L-[4,5-³H]leucine/ml. Incubation media were not supplemented with additional amino acids, since incorporation of [³H]leucine into proinsulin and insulin remained linear with time over a 3 h incubation period. The tubes were then incubated with gentle shaking for 3 h at 37°C. After incubation, the islets were rinsed twice in phosphate-buffered saline (40 mM-NaH₂PO₄/0.9% NaCl), pH 7.4, containing 1 mg of non-labelled leucine/ml, and subsequently sonicated for 15 s (M.S.E. Ultrasonic Disintegrator; high power, amplitude 3) in 150 μ l of phosphate-buffered saline. Samples (10 μ l) of the sonicated preparation were taken for determination of protein content (Lowry *et al.*, 1951), with crystalline bovine albumin as standard.

Coupling of insulin antibodies to CNBr-activated Sepharose 4B. The procedure for coupling followed the method of Berne (1975). Anti-insulin serum or normal guinea-pig serum was allowed to react with

CNBr-activated Sepharose 4B for 3 h with constant mixing at room temperature (22°C). After washing in the coupling buffer, the Sepharose beads were incubated in 1 M-ethanolamine to block any remaining active groups. A washing cycle, alternating between 0.1 M-sodium acetate, pH 4.0, and 0.1 M-sodium borate, pH 8.0, buffers, removed any non-covalently adsorbed proteins. Finally the AIS-Sepharose [(anti-insulin serum)-Sepharose] and the NGS-Sepharose [(normal guinea-pig serum)-Sepharose] were resuspended in phosphate-buffered saline (10 mg/100 μ l) and the binding capacity of the suspension of AIS-Sepharose was measured with iodinated ox insulin. The AIS- and NGS-Sepharose could be stored for several months at 4°C with no apparent loss of activity.

Determination of proinsulin and insulin biosynthesis. Samples (20 μ l) of the diluted preparations of sonicated islets were mixed with 1 ml of phosphate-buffered saline, supplemented with 2 mg of bovine albumin/ml, and containing 20 mg of either AIS-Sepharose or NGS-Sepharose, by the method of Berne (1975). The NGS-Sepharose served as a control for non-specific binding of labelled proteins, specific binding being the difference between the radioactivity in the material bound to the AIS- and NGS-Sepharose beads. The immune-binding reaction was carried out in polythene tubes rotated end-over-end (20 rev./min) for 90 min at room temperature. The Sepharose beads were then washed four times with phosphate-buffered saline and solubilized overnight in 0.8 ml of Soluene 350 tissue solubilizer.

Determination of total islet protein biosynthesis. Phosphate-buffered saline (50 μ l) supplemented with 5 mg of bovine serum albumin/ml was mixed with 25 μ l of sonicated islet preparation and the proteins were precipitated by the addition of 2 ml of 5% (w/v) trichloroacetic acid containing 1 mg of non-labelled leucine/ml. The resulting precipitate was collected by centrifugation (10 min at 2000 g), then washed twice with 5% trichloroacetic acid and finally solubilized overnight in 0.3 ml of Soluene.

Determination of radioactivity. After digestion in Soluene, the radioactivity of each sample was determined by using a Beckman LS233 liquid-scintillation counter. The scintillator solution used was toluene/PPO/dimethyl-POPOP (1000:5:0.5, v/w/w).

Chromatographic characterization of labelled Sepharose-bound material. Labelled proteins were extracted from the Sepharose beads with 2 M-acetic acid, supplemented with bovine albumin and carrier ox insulin, and chromatographed on a Sephadex G-50 column (1 cm $\times$ 60 cm) in 2 M-acetic acid. Fractions (1 ml) eluted from the column were collected and their A₂₈₀ was recorded. A 0.1 ml sample of each fraction was added to 1 ml of toluene/Triton/PPO (700:300:3, v/v/v) for liquid-scintillation counting.

Expression of results

Incorporation of radioactivity was expressed as c.p.m./ μ g of islet protein, which corrects for any differences in size and content of the islets isolated from normal, pregnant and starved rats. Furthermore, by expressing insulin biosynthesis as a ratio of the mean control value at the glucose concentration of 2 mM found in normal islets, which were included in each experiment, we were able directly to compare results from different series of experiments.

Results

Use of AIS- and NGS-Sephacrose 4B

The use of anti-insulin serum coupled to a solid phase (Sephacrose 4B) greatly facilitated the separation of newly labelled proinsulin and insulin from other labelled islet proteins, separation being achieved by a single incubation followed by a short centrifugation and washing of the bound protein material. The efficiency of this procedure for monitoring changes in the rate of biosynthesis of proinsulin and insulin is governed by the relative binding capacity of the antisera for proinsulin and insulin; complete binding of proinsulin can, however, be achieved by addition of excess antibody. Preliminary binding-capacity studies with sonicated islet samples, to which tracer amounts of 125 I-labelled insulin [prepared by the chloramine-T method of Hunter & Greenwood (1962) adapted as described by Green (1973)] were added, showed a complete binding of all the insulin-immunoreactive material to AIS-Sephacrose, as was shown by Berne (1975) using a similar method. This procedure has been used throughout the present study.

Groups of 20 islets isolated from normal female rats were incubated with [3 H]leucine for 3 h in the presence of 20 mM-glucose. The islets were sonicated, a sample of the sonicated material was made to react with AIS- or NGS-Sephacrose 4B and the bound protein material extracted as described under 'Methods'. The elution profiles of the extracted proteins, fractionated on a Sephadex G-50 column, are shown in Fig. 1. The insulin-antibody-reactive material extracted from AIS-Sephacrose showed three distinct peaks of radioactivity. The first peak accompanied albumin in the void volume, the third peak was co-eluted with the ox insulin marker, and the second peak appeared in a region which in calibrations of the column corresponded to a pro-insulin marker (Fig. 1). For material bound to NGS-Sephacrose there was a peak of radioactivity with the void volume, but almost a complete absence of radioactivity from the proinsulin or insulin regions (Fig. 1). The radioactivity bound to the AIS-Sephacrose beads generally exceeded the non-specific adsorption on the NGS-Sephacrose by 2-5-fold with extracts of islets that had been incubated in low and high glucose respectively.

Role of cyclic AMP in the regulation of insulin biosynthesis in pregnancy

To study the effects of glucose on the rates of biosynthesis of proinsulin and insulin in normal and pregnant rats, incubations of isolated islets of Langerhans were performed in the presence of various concentrations of glucose. The dose-response curves for insulin biosynthesis of islets from normal female and pregnant rats and normal islets incubated in the presence of 3-isobutyl-1-methyl-

Fig. 1. Gel filtration of radioactive islet proteins dissociated from the AIS- and NGS-Sephacrose 4B

Normal rat islets which had been incubated for 3 h in 20 mM-glucose were sonicated. After incubation of portions of the sonicated preparation with AIS- or NGS-Sephacrose 4B, the beads were washed with phosphate-buffered saline and finally extracted with 2 M-acetic acid. The extracts were supplemented with bovine serum albumin and carrier ox insulin and fractionated on a Sephadex G-50 column (1 cm $\times$ 60 cm) by using 2 M-acetic acid (fraction size 1 ml). Details of the incubations, immune binding and gel chromatography are given in the Experimental section. —, 3 H-labelled proteins extracted from AIS-Sephacrose 4B; ····, 3 H-labelled proteins extracted from NGS-Sephacrose 4B; ----, A_{280} .

Fig. 2. *Insulin-biosynthetic response of islets isolated from normal female and pregnant rats to 2–20mm-glucose and the response of normal islets to glucose plus 3-isobutyl-1-methylxanthine*

Mean values ($\pm$ S.E.M.) for insulin biosynthesis are expressed as a ratio of the mean control value found in normal islets during each experiment at a glucose concentration of 2mm (204 ± 26 c.p.m./ μ g of islet protein). Insulin-biosynthetic responses of the isolated islets were determined as described in the text. ●, Islets from normal female rats; ○, islets from 19-day-pregnant rats; ■, islets from normal rats incubated in the presence of glucose + 3-isobutyl-1-methylxanthine (0.5mm).

xanthine are shown in Fig. 2, in which for simplicity all values of amino acid incorporation are compared with the value observed in untreated rats at a glucose concentration of 2mm in each experiment. The islets isolated from the pregnant rats showed an increased rate of synthesis in the presence of 2mm-glucose as compared with the normal ones, and indeed there was a significantly greater increase in incorporation at all the glucose concentrations studied (Fig. 2). Addition of 3-isobutyl-1-methylxanthine significantly increased the biosynthetic response of normal islets over the glucose-concentration range 6–20mm; however, there appeared to

Table 1. *Insulin-biosynthetic response of islets isolated from normal female and progesterone-injected rats to 2–6mm-glucose or glucose plus 3-isobutyl-1-methylxanthine*
Mean values of approximately eight observations $\pm$ S.E.M. for insulin biosynthesis are given. Details of steroid treatment and incubation procedures are given in the text.

Addition to medium	$10^{-1} \times$ Insulin biosynthesis (c.p.m./ μ g of total islet protein)	
	Islets of control rats	Islets of progesterone-injected rats
Glucose (2mm)	18.8 ± 2.5	18.1 ± 2.1
Glucose (6mm)	68.0 ± 1.5	71.6 ± 2.9
Glucose (2mm) + 3-isobutyl-1-methylxanthine (0.5mm)	18.9 ± 2.8	23.3 ± 4.5
Glucose (4mm) + 3-isobutyl-1-methylxanthine (0.5mm)	45.9 ± 2.4	$65.6 \pm 2.2^*$
Glucose (6mm) + 3-isobutyl-1-methylxanthine (0.5mm)	89.4 ± 6.0	$124.7 \pm 6.4^*$

* Values significantly different from controls ($P < 0.01$) by Student's *t* test.

be no stimulatory effect of increased cyclic AMP concentrations at low glucose concentrations (2 or 4mm) (Fig. 2).

Treatment of rats with progesterone

Islets were isolated from steroid-treated and control animals after 10 days and incubated in the presence of 2–6mm-glucose or glucose plus 3-isobutyl-1-methylxanthine: the pattern of amino acid incorporation into proinsulin/insulin is shown in Table 1. There appeared to be no marked alteration in insulin biosynthesis in the steroid-injected rats in the presence of 2mm- or 6mm-glucose; however, in the presence of 4mm- or 6mm-glucose plus 3-isobutyl-1-methylxanthine there was a significant increase in insulin biosynthesis in islets isolated from the progesterone-injected animals.

Insulin biosynthesis in starved rats

The pattern of insulin biosynthesis during incubation of islets from starved or normal rats in the presence of 2–20mm-glucose is shown in Fig. 3; all values of amino acid incorporation are again expressed as a ratio of the value observed in normal rats at a glucose concentration of 2mm in each experiment. The biosynthetic response of islets isolated from rats starved for 48h was significantly diminished over the glucose concentrations 4–20mm (Fig. 3). Incubation of islets from starved rats with 0.5mm-3-isobutyl-1-methylxanthine markedly increased their insulin-biosynthetic response to 2–20mm-glucose and restored the pattern of insulin biosynthesis to resemble that seen in islets from fed rats, at least in the presence of 4mm-, 6mm- or 8mm-glucose.

Fig. 3. Insulin-biosynthetic response of islets isolated from normal female and starved rats to 2–20mm-glucose and the synthetic response of starved islets to glucose plus 3-isobutyl-1-methylxanthine

Mean values ($\pm$ S.E.M.) for insulin biosynthesis are expressed as a ratio of the mean control value found in normal islets during each experiment at a glucose concentration of 2mm (191 ± 31 c.p.m./ μ g of islet protein). Insulin-biosynthetic responses of the isolated islets were determined as described in the text. ●, Islets from normal female rats; ▲, islets from starved rats; △, islets from starved rats incubated in the presence of glucose + 3-isobutyl-1-methylxanthine (0.5mm).

Fig. 4. Effect of glucose on [3 H]leucine incorporation into trichloroacetic acid-precipitable proteins of islets isolated from normal, pregnant and starved rats

Mean values ($\pm$ S.E.M.) for incorporation of 3 H into trichloroacetic acid-precipitable islet proteins are expressed as a ratio of the mean control value found in normal islets during the same experiment at a glucose concentration of 2mm (4440 ± 180 c.p.m./ μ g of islet protein). Total protein biosynthesis of the isolated islets was determined as described in the text. ●, Islets from normal female rats; ○, islets from 19-day-pregnant rats; ▲, islets from starved rats.

Total protein biosynthesis

To investigate possible alterations in amino acid incorporation into trichloroacetic acid-precipitable islet proteins during pregnancy and starvation, islets isolated from normal, pregnant and starved rats were incubated in the presence of 2–20mm-glucose. Rates of incorporation were compared with the values obtained in normal rats at a glucose concentration of 2mm in each experiment. There was no significant difference in incorporation between islets isolated from normal and starved rats in the presence of 2–20mm-glucose, both showing a 1.6-fold increase in [3 H]leucine incorporation (Fig. 4); however, islets isolated from pregnant rats showed an increased rate of incorporation at high glucose concentrations (20mm) and an overall 2-fold increase

in radioactive amino acid incorporation over the glucose concentration range 2–20mm (Fig. 4).

Discussion

It is now well established that glucose, in addition to strongly stimulating insulin release from β -cells, is also capable of stimulating insulin biosynthesis (Howell & Taylor, 1966; Lin & Haist, 1969; Tanese *et al.*, 1970), and this was confirmed in the present experiments by using the relatively new Sepharose procedure, in which an 8–9-fold stimulation was seen on increasing the glucose concentration from 2 to 20mm in fed rats. This compares favourably with the findings of other workers (Pipeleers *et al.*, 1973;

Berne, 1975). A similar increase in glucose concentration has been shown here to stimulate the incorporation of amino acids into total islet proteins to a much smaller extent (1–2-fold), in accordance with previous observations (Berne, 1975; Andersson, 1976; Tijoe & Bouman, 1976). The stimulatory effect of glucose on protein synthesis in the β -cell is therefore selective for proinsulin biosynthesis. The effect is very rapid and does not depend on the synthesis of new mRNA; it rather appears to be due to selective translational enhancement (Morris & Korner, 1970; Permutt & Kipnis, 1970; Steiner *et al.*, 1972).

Relationship between insulin secretion and biosynthesis in pregnancy

There have been numerous reports (Spellacy & Goetz, 1963; Kalkhoff *et al.*, 1964; Campbell *et al.*, 1971) that indicate an enhanced insulin secretion in late human pregnancy. In addition, stimulation by glucose of insulin secretion from isolated islets of Langerhans from pregnant rats gives rise to hypersecretion of insulin (Costrini & Kalkhoff, 1971; Green & Taylor, 1972), so it seemed possible that an enhanced rate of biosynthesis could occur simultaneously with this increased secretory response to glucose. The present results appear to support this hypothesis; the dose-response curve of biosynthesis to glucose in pregnancy shows an increased basal rate of synthesis as compared with the normal rat, and a significant increase in amino acid incorporation at all the glucose concentrations studied (see also Bone & Taylor, 1976).

Total protein biosynthesis was also increased over this glucose concentration range, the increase being only 2-fold, compared with the 10-fold stimulation of insulin biosynthesis. Such a mechanism presumably explains why the insulin content of islets from pregnant rats is unchanged or even increased after a period of insulin hypersecretion.

Green *et al.* (1973) suggested that the higher cyclic AMP concentrations observed in islets in pregnancy may be responsible for their greater insulin-secretory responsiveness to glucose stimulation. Studies on the actions of theophylline and cyclic AMP on biosynthesis of insulin have indicated that the cyclic AMP system may also be involved in the regulation of insulin biosynthesis in normal rat islets (Schatz *et al.*, 1973). To investigate a possible involvement of the cyclic AMP system in the enhanced insulin biosynthesis observed in pregnancy, islets from normal female rats were incubated in increasing concentrations of glucose or glucose plus 3-isobutyl-1-methylxanthine, a phosphodiesterase inhibitor which is known to increase the cyclic AMP content of isolated islets of normal (Montague & Cook, 1971) and pregnant (Green *et al.*, 1973) rats.

There is no evidence that 3-isobutyl-1-methylxanthine exerts its effects in islets by any mechanism other than by inhibition of phosphodiesterase activity. Elevation of cyclic AMP concentrations significantly increased the insulin-synthetic response of normal islets over the glucose concentration range 5–20 mM, which is consistent with a possible involvement of the cyclic AMP system in the potentiation of the increased glucose-stimulated insulin biosynthesis over this concentration range which was observed in pregnancy. Such an increase in cyclic AMP would not, however, account for the observed increase in basal rates of insulin synthesis in pregnancy.

Changes of considerable magnitude are known to occur in the blood concentrations of other hormones during pregnancy, and some of these changes have been shown to affect insulin-secretory activity, for instance the relative effects of pregnancy, oestradiol and progesterone on plasma insulin and islet insulin secretion (Costrini & Kalkhoff, 1971). To investigate possible changes in insulin-biosynthetic activity due to the elevated concentrations of progesterone observed in late rat pregnancy (Bartholomeusz *et al.*, 1976), female rats were injected with a long-acting progestogen, and after 10 days we observed radioactive amino acid incorporation into proinsulin and insulin in islets isolated from the steroid-treated and control animals. Over the glucose concentration range 2–6 mM there was no marked alteration in insulin biosynthesis; however, in the presence of 4 mM- or 6 mM-glucose + 3-isobutyl-1-methylxanthine there was a significant increase in insulin biosynthesis in the progestogen-injected rats. These results suggested that the increased progesterone concentrations seen in late pregnancy in rats (Bartholomeusz *et al.*, 1976) could in the long term possibly contribute to the increase in glucose-stimulated insulin biosynthesis. The increased basal rates of insulin biosynthesis observed in pregnancy remain unexplained.

Relationship between insulin secretion and biosynthesis in starvation

Starvation has been shown to impair markedly the ability of glucose to stimulate insulin secretion in the rat, and this decrease in secretion is not reflected in a change in islet insulin content (Malaisse *et al.*, 1967; Grey *et al.*, 1970a). The defect does not appear to involve the secretory mechanism itself, since the response to glucose seen in starving rats may be rapidly overcome by injection of theophylline (Grey *et al.*, 1970b) or by addition of theophylline to the medium in which islets from starved rats are incubated (Howell *et al.*, 1973). This evidence, together with the observed decreases in cyclic AMP concentrations (Selawry *et al.*, 1972, 1973) and also in adenylate cyclase activity (Howell *et al.*, 1973) in isolated islets of Langerhans from starved rats,

is consistent with the concept that the alterations of cyclic AMP concentrations within the β -cell may be responsible for the decreased secretory response to glucose seen after starvation.

So far, however, there is limited knowledge about the effects of starvation on rates of insulin biosynthesis. Results shown above (Fig. 3) suggest that starvation for 48 h significantly decreased the rate of amino acid incorporation into proinsulin and insulin over the glucose concentration range 4–20 mM, but the labelling of trichloroacetic acid-precipitable proteins remained unchanged. Similar results have been reported by Tijoe & Bouman (1976) in a study of starvation (24 and 72 h), and they suggest a selective inhibition of insulin biosynthesis in rat islets under those conditions. However, Andersson (1976) showed no difference in the rate of glucose-stimulated insulin biosynthesis between islets isolated from fed or starved mice, and this is consistent with the absence of a fall in cyclic AMP content in islets from starved mice (Capito & Hedekov, 1974). It has been suggested that starvation may increase the breakdown of islet proteins (Gylfe, 1974), thereby increasing the intracellular free amino acid pool, and this could lead to a dilution of the free [^3H]leucine pool and decreased incorporation of label into proinsulin. However, this possibility of isotope dilution seems unlikely in view of the lack of effect of starvation on incorporation into trichloroacetic acid-precipitable proteins.

Incubation of islets from starved rats in the presence of glucose and 3-isobutyl-1-methylxanthine restored the pattern of insulin biosynthesis to resemble that seen in islets from fed rats, except at high glucose concentrations (20 mM) where rates of insulin biosynthesis remained one-third below fed values. These findings closely parallel those of Howell *et al.* (1973) in a study of the regulation of insulin secretion in starved rats. It seems possible that, as with insulin secretion, alterations in cyclic AMP concentrations could be at least partially responsible for the altered biosynthetic response to glucose after a period of starvation.

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