Effects of a diabetogenic strain of encephalomyocarditis (EMC) virus on protein synthesis in mouse islets of Langerhans

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The effects of a diabetogenic strain of encephalomyocarditis (EMC) virus on total protein and insulin biosynthesis in mouse islets of Langerhans have been studied in tissue culture. In dispersed mouse islets, the rates of protein biosynthesis were assessed by measuring the incorporation of [³H]leucine into proteins. In infected dispersed islets incubated in 20 mM-glucose, both insulin and total protein biosynthesis were decreased at 6 h; only insulin biosynthesis was significantly decreased at 3 h. In whole islets, EMC virus brought about a decrease in glucose-stimulated protein and insulin biosynthesis as early as 2 h after infection without concomitant effects on insulin release. This inhibition of protein biosynthesis was still apparent at 20 h post-infection, at which time insulin release was found to be markedly elevated, and the islet insulin content was moderately decreased. At 44 h post-infection, glucose-induced insulin biosynthesis was preferentially inhibited. Infected islets at this later time point also displayed elevated levels of insulin release, and a marked loss of islet insulin content. When insulin mRNA and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA levels were assessed by dot-blot hybridization using appropriate cDNA probes, levels of insulin mRNA were shown to decrease steadily during the first 20 h of infection, in contrast with the levels of GAPDH mRNA. At 44 h post-infection, both types of mRNA were markedly decreased. It is suggested that there is an initial early 'shut-off' of protein synthesis without other detectable changes in islet function. This is followed by a phase where both insulin mRNA levels and insulin synthesis are dramatically decreased.

INTRODUCTION

A number of viruses are now known to induce diabetes in animals, and because of their potentially important relationship to human diabetes, there has been much interest in the way in which they may alter biochemical functions in the islets of Langerhans. Thus effects on the release and synthesis of insulin have been examined in Cocksackie B4-infected islets in tissue culture [1], as well as in whole animals inoculated with this virus [2,3].

Another picornavirus, encephalomyocarditis (EMC) virus, also induces a diabetes-like syndrome in susceptible mice [4,5]. EMC virus precipitates this disease by infecting and destroying pancreatic β -cells, and the degree of hyperglycaemia produced correlates well with the amount of β -cell damage [6]. Long-term biochemical changes in islets isolated from infected mice have also been reported, and insulin biosynthesis was shown to be impaired at 60 days post-infection [7]. Moreover, when isolated islets were infected *in vitro*, there was a marked inhibition of insulin biosynthesis from 2 days onwards, which did not appear to be due to simple cell destruction [8]. EMC virus has also been shown to inhibit insulin biosynthesis as early as 6 h post-infection in islets isolated from infected mice [9]. Nevertheless, there has not so far been any systematic study of the changes induced by EMC virus on protein synthesis in islets, particularly at short periods of time after infection.

The aim of this work was to study further the early impairment of insulin biosynthesis observed after EMC virus infection of isolated islets *in vitro*. Evidence is presented here that EMC virus induces an early 'shut-off' of glucose-induced insulin biosynthesis, which is followed by a dramatic loss of insulin mRNA in conjunction with a selective impairment of glucose-stimulated insulin biosynthesis prior to substantial β -cell lysis.

EXPERIMENTAL

Materials

L-[4,5-³H]Leucine (120–190 Ci/mmol), ¹²⁵I-insulin, [α -³²P]-dCTP (3000 Ci/mol) and a Multiprime DNA labelling system were purchased from Amersham International, Amersham, Bucks., U.K. A Genescreen hybridization transfer membrane was obtained from NEN Research Products, Boston, MA, U.S.A. Formamide and phenol were from Gibco, and were of redistilled nucleic acid grade. Collagenase (Type 1A) and BSA (Fraction V) were from Sigma Chemical Co., Poole, Dorset, U.K. Heat-inactivated foetal calf serum (FCS) was from Flow Laboratories, Irvine, Scotland, U.K. The myocardial (M) strain of EMC virus was donated by Dr. D. R. Gamble, Public Health Laboratory, West Park Hospital, Epsom, U.K. The cDNA probe to insulin (pRI-7) was a gift from Professor C. Hellerström, Uppsala University, Uppsala, Sweden, and the cDNA probe (pRGAPDH-13) to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was donated by Dr. M. Timmers, Leiden University, The Netherlands.

Virus preparation

Virus was prepared by serial passage through 3-week-old male DBA₂ mice, using a heart suspension as the inoculum. The mouse heart tissue was harvested at the second and third passage and homogenized in Hanks' salt solution to yield a 10% (w/v) homogenate. After freeze-thawing the heart homogenates three times to release virus, the virus suspension was clarified by centrifugation at 1000 g for 10 min. Portions of the virus were then stored at –20 °C, and virus was assayed for plaque-forming units (p.f.u.) on L929 cells. For controls, a 10% heart homogenate was prepared from age- and sex-matched non-infected mice, and then treated as described.

Abbreviations used: EMC virus, encephalomyocarditis virus; FCS, foetal calf serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; p.f.u., plaque-forming units; PBS, phosphate-buffered saline.

Islet isolation and dispersion

Islets were isolated from collagenase-digested pancreas of 8–10-week-old male DBA₂ mice [10]. Isolated islets were then either maintained in tissue-culture medium RPMI 1640 supplemented with 10% FCS, or dispersed into a single cell suspension before infection with the virus [11].

Studies on EMC-infected dispersed islets

Freshly isolated islets were dispersed [11], washed in RPMI 1640 and inoculated with EMC virus at 5 p.f.u./cell in a minimum volume of buffer. Virus was allowed to adsorb on to the cells over 30 min at room temperature. Cells were then washed twice in RPMI 1640 (10% FCS), placed in 1.5 ml tubes at 3×10^4 cells/tube, and incubated in 1.0 ml of this medium for 3 or 6 h in a CO₂ incubator at 37 °C in a humidified atmosphere gassed with air/CO₂ (19:1) [12]. Cells were then washed with bicarbonate buffer [13] containing 0.1% BSA and 2 mM-glucose, and then resuspended in 100 μ l of buffer which contained L-[4,5-³H]leucine (50 μ Ci/ml) and glucose (2 or 20 mM) and incubated for 45 min at 37 °C. Cells were then washed with 1.0 ml of ice-cold phosphate-buffered saline (PBS) containing leucine (10 mM), resuspended in 60 μ l of PBS and sonicated for 15 s. Portions of the sonicated samples were used to determine rates of insulin biosynthesis by a Protein A–Sepharose technique [14], and total protein biosynthesis by measuring radioactivity in trichloroacetic acid precipitates of islets [15]. Due to the cross-reactivity of the anti-insulin antibody with pro-insulin, the incorporation of [³H]leucine into insulin is denoted by (pro)insulin (PI–I). DNA content was determined by a fluorimetric assay [16].

Studies on EMC-infected whole islets

Groups of 10 islets were placed in Microfuge tubes, and after washing once in RPMI 1640 islets were inoculated with EMC virus at 10^4 p.f.u./islet in a minimum volume of buffer. Virus was allowed to adsorb to the islets for 1 h at 37 °C. Islets were then washed twice in RPMI 1640 (+10% FCS) and incubated in this medium for 2, 20 or 44 h in a CO₂ incubator as described above. Islets were then washed with bicarbonate buffer [13] containing 0.1% BSA and 2 mM-glucose, resuspended in 1.0 ml of buffer and incubated for 30 min at 37 °C; then resuspended in 100 μ l of buffer containing L-[4,5-³H]leucine (50 μ Ci/ml) and glucose (2 or 20 mM) and incubated for a further 2 h at 37 °C. The supernatant was removed for analysis of insulin release by radioimmunoassay [17]. Islets were then washed with 1.0 ml of ice-cold PBS containing leucine (10 mM), resuspended in 100 μ l of 0.003 M-HCl and sonicated for 15 s. Aliquots of the sonicated samples were used to determine the rates of biosynthesis of insulin and of total protein [14,15]. Insulin content of the sonicated samples was determined by radioimmunoassay [17].

In a further series of experiments, up to 600 islets were inoculated with EMC virus at 10^4 p.f.u./islet and virus was allowed to adsorb to the islets for 1 h at room temperature. Islets were then washed twice in RPMI 1640 and incubated in RPMI 1640 with 10% FCS for 12, 20, 33 or 44 h in a CO₂ incubator. Groups of 50–100 islets were then washed in ice-cold PBS and total cellular RNA was extracted by sonicating the islets in 400 μ l of 200 mM-Tris (pH 9.0)/100 mM-LiCl/25 mM-EDTA/1% SDS and 400 μ l of phenol/chloroform/isoamyl alcohol/2-mercaptoethanol/8-hydroxyquinoline (24:24:2:0.2:0.1, by vol.). The mixture was then re-extracted with the solvent mixture followed by ethanol precipitation of the RNA [20]. Insulin mRNA and GAPDH mRNA were then quantified by dot-blot analysis [18,19].

Measurement of insulin mRNA and GAPDH mRNA

RNA from 50–100 islets was denatured for 1 h at 50 °C in 10 μ l of 1 M-glyoxal/50% (v/v) dimethyl sulphoxide/10 mM-sodium phosphate (pH 7.0), diluted with 4 vol. of 20 $\times$ SSC (SSC = 0.15 M-NaCl/0.015 M-sodium citrate) and bound to a Genescreen hybridization membrane using a suction apparatus [20]. After prehybridization, the membranes were hybridized at 42 °C with the appropriate [α -³²P]dCTP-labelled probe [21,22]. After hybridization, membranes were washed in stringent conditions (0.1 $\times$ SSC/0.1% SDS at 50 °C) and exposed at –70 °C to Kodak XAR-5 film (Kodak, Rochester, NY, U.S.A.) with an intensifying screen. The dot intensities were then measured by densitometry. Northern blot analysis showed that the insulin cDNA probe was selective for insulin mRNA, and that the extracted insulin mRNA was intact. Moreover, the insulin cDNA probe was shown to be selective in that it failed to hybridize to dot-blot of total cellular RNA extracted from mouse fibroblast cells, and the densitometric signal of dot-blot was found to correlate with islet cell number when RNA extracts of between 20 and 80 islets were assessed (linear regression = 0.987) (see Fig. 1). The GAPDH cDNA probe was found to be selective for GAPDH mRNA, and linearity between signal and islet number was shown.

EMC virus replication in islets

Isolated islets were inoculated as described earlier, and virus was allowed to adsorb for 1 h at room temperature. Duplicate groups of 100 islets were then incubated in a CO₂ incubator in 3.0 ml of RPMI 1640 (10% FCS) for 0, 6, 20 or 44 h. Islets were then freeze–thawed twice, clarified by filtration through a 0.2 μ m pore size nylon membrane and assayed on L929 cells for virus (p.f.u.).

Light microscopy studies

Islets were infected as above and incubated in RPMI 1640 (10% FCS) for up to 44 h post-infection. At 20, 33 and 44 h post-infection, islets were removed and stained with Trypan Blue to check viability. Islets were then viewed against a white background using a dissecting microscope at $\times 30$ magnification.

Statistical analysis of data

All significant differences were determined using Student's *t* test. Results are presented as means $\pm$ S.E.M., with the numbers of observations per group in parentheses.

Fig. 1. RNA dot-blot analysis of insulin mRNA in total cellular RNA extracts from mouse islets and mouse fibroblasts (L929 cells)

RNA was extracted from freshly isolated islets (20–80) and hybridized with the [α -³²P]dCTP-labelled insulin cDNA probe, pRI-7, as described in the text. To determine the specificity of the pRI-7 cDNA probe for insulin mRNA under the hybridization conditions employed, dot-blot analysis was also performed on total cellular RNA extracted from freshly harvested L929 cells (up to 4.8×10^5 cells, equivalent to approx. 200 islets). Islet and L929 cell autoradiographic signals are illustrated; a, b, c and d correspond to 80, 60, 40 and 20 islets respectively, whereas e and f correspond to 4.8×10^5 and 2.4×10^5 L929 cells respectively.

Table 1. Effects of glucose on (pro)insulin and total protein biosynthesis in dispersed islets infected with EMC virus

Freshly isolated islets were dispersed and then infected with EMC virus at 5 p.f.u./cell (see the text). Incorporation of L-[4,5-³H]leucine into (pro)insulin (PI-I) was measured by an immunoadsorption technique (see the text). Incorporation into total protein was determined in trichloroacetic acid precipitates (TCA). The percentage contribution of labelled (pro)insulin to the total pool of labelled islet proteins is denoted by (PI-I/TCA) × 100. Results are expressed as means ± s.e.m., with the numbers of observations per group in parentheses. Results where *P* < 0.05 for differences between experimental values and equivalent controls are denoted by *, or where *P* < 0.01 by **. Differences from equivalent observations at 2 mM, where *P* < 0.01, are shown by †.

Time post-infection (h)	Glucose concn. (mM)	PI-I (c.p.m./45 min per ng of DNA)		TCA (c.p.m./45 min per ng of DNA)		(PI-I/TCA) × 100 (%)	
		Control	Infected	Control	Infected	Control	Infected
3	2	1.3 ± 0.3 (9)	1.8 ± 0.7 (8)	35.7 ± 5.6 (9)	48.5 ± 12.1 (9)	3.6 ± 0.6 (9)	2.2 ± 0.6 (8)
3	20	12.4 ± 1.5 (10)†	7.6 ± 1.3 (8)*†	93.5 ± 16.2 (10)†	78.7 ± 18.1 (9)	13.6 ± 1.6 (9)†	12.0 ± 2.0 (9)†
6	2	5.8 ± 0.6 (16)	4.1 ± 0.5 (16)*	48.4 ± 6.5 (16)	50.6 ± 7.3 (17)	15.7 ± 1.1 (16)	11.3 ± 1.0 (17)**
6	20	12.9 ± 1.1 (18)†	8.3 ± 1.0 (18)**†	63.9 ± 6.3 (19)	42.1 ± 4.7 (18)**	23.4 ± 1.1 (18)†	20.5 ± 2.0 (18)†

RESULTS AND DISCUSSION

Previous studies have shown that the myocardial (M) strain of EMC virus can induce an early dysfunction in insulin biosynthesis in infected β -cells in the absence of prominent cell lysis [8]. The results presented here confirm and expand these earlier findings. As is well known, glucose stimulates insulin biosynthesis at concentrations above 2–4 mM [23]. Moreover, the biosynthesis of insulin rather than that of other islet proteins is preferentially affected by glucose [24]. However, when a dispersed cell preparation of islets was infected with EMC virus, the induction of insulin biosynthesis was decreased (Table 1). Later, at 6 h post-infection, both insulin biosynthesis and total protein biosynthesis were inhibited at high glucose concentrations. Similar results were also found in infected islet cells that had been dispersed using a different method [25] (T. Ward, M. J. Clemens & K. W. Taylor, unpublished work). At 6 h post-infection, insulin biosynthesis was also found to be decreased in the infected islets at the low (2 mM) glucose concentration.

Precisely how this virus interferes with protein synthesis in β -cells is a matter of conjecture. The observed early inhibition of glucose-stimulated insulin biosynthesis may be a consequence of virus-mediated shut-off of protein biosynthesis, which has been shown to occur in some cells [26] and with other viruses. This process may involve a change in the state of phosphorylation or the subcellular distribution of one or more of the initiation

factors [27,28], induction of a translation inhibitor [29], or alterations in intracellular ionic conditions [30,31].

In order to study the later effects of EMC virus infection on islet cell protein biosynthesis, whole islets were infected *in vitro* and [³H]leucine incorporation into insulin and other islet cellular proteins was measured at intervals up to 44 h post-infection. At 2 h post-infection, a moderate decrease in protein biosynthesis was observed only in islets stimulated by high glucose concentrations (Table 2); this correlates with the early inhibition of protein biosynthesis described above. Later, at 20 h post-infection, the moderate decrease in glucose-stimulated protein biosynthesis was still apparent. At 44 h post-infection, both total protein biosynthesis and insulin biosynthesis were dramatically decreased by virus infection, and this inhibition was again selective toward glucose-induced insulin biosynthesis.

The possibility that the inhibition of protein biosynthesis might have been non-specific was also investigated. Thus host anti-viral factors such as the interferons, which are capable of inhibiting protein biosynthesis, could be present in the EMC heart inoculum. However, when virus was removed from the inoculum by ultracentrifugation (140 000 g, 1 h), the supernatant used had no effect on protein biosynthesis (T. Ward, M. J. Clemens & K. W. Taylor, unpublished work). It is therefore concluded that the observed inhibition of protein biosynthesis is likely to be due to EMC virus infection.

At 2 h post-infection, insulin release from EMC-infected islets

Table 2. Effects of glucose on (pro)insulin and total protein biosynthesis in whole islets infected with EMC virus

Freshly isolated islets were infected with EMC virus at 10⁴ p.f.u./islet (see the text). Control and infected islets were then cultured in RPMI 1640 (10% FCS) for 2, 20 and 44 h. Incorporation of L-[4,5-³H]leucine into (pro)insulin (PI-I) was measured by an immunoadsorption technique (see the text). Incorporation into total protein was determined in trichloroacetic acid precipitates (TCA). The percentage contribution of labelled (pro)insulin to the total pool of labelled islet proteins is denoted by (PI-I/TCA) × 100. Results are expressed as means ± s.e.m., with the numbers of observations per group in parentheses. Results where *P* < 0.05 for differences between experimental values and equivalent controls are denoted by *, or where *P* < 0.01 by **. Differences from equivalent observations at 2 mM, where *P* < 0.02, are shown by †.

Time post-infection (h)	Glucose concn. (mM)	PI-I (c.p.m./h per islet)		TCA (c.p.m./h per islet)		(PI-I/TCA) × 100 (%)	
		Control	Infected	Control	Infected	Control	Infected
2	2	17 ± 2 (6)	12 ± 3 (6)	1090 ± 131 (6)	1060 ± 76 (6)	1.6 ± 0.2 (6)	1.2 ± 0.2 (6)
2	20	249 ± 19 (7)†	190 ± 22 (9)*†	2391 ± 128 (8)†	1807 ± 102 (9)*†	11.7 ± 1.4 (7)†	10.6 ± 1.1 (9)†
20	2	107 ± 12 (10)	88 ± 10 (9)	1794 ± 73 (9)	1573 ± 120 (9)	5.8 ± 0.7 (9)	5.6 ± 0.3 (9)
20	20	386 ± 35 (9)†	297 ± 10 (10)*†	2472 ± 113 (10)†	2134 ± 89 (9)*†	17.3 ± 1.9 (9)†	13.5 ± 0.6 (9)†
44	2	25 ± 6 (5)	11 ± 2 (5)	753 ± 81 (5)	563 ± 19 (5)	3.1 ± 0.5 (5)	1.8 ± 0.3 (5)
44	20	345 ± 23 (5)†	28 ± 4 (5)**†	1730 ± 57 (5)†	601 ± 37 (5)**	20.0 ± 1.5 (5)†	4.6 ± 0.4 (5)**†

Table 3. Insulin release and insulin content of whole islets infected with EMC virus

Freshly isolated islets were infected and incubated as described in the legend to Table 2. Insulin released into the medium was measured by radioimmunoassay. Insulin content was measured by radioimmunoassay of islets sonicated in 0.003 M-HCl. Results are expressed as means $\pm$ S.E.M., with the numbers of observations per group in parentheses. Results where $P < 0.02$ for differences between experimental values and equivalent controls are denoted by *. Differences from equivalent observations at 2 mM, where $P < 0.05$, are shown by †, and where $P < 0.001$ by ††.

Time post infection (h)	Glucose concn. (mM)	Insulin release (pg/min per islet)		Insulin content (ng/islet)	
		Control	Infected	Control	Infected
2	2	0.9 $\pm$ 0.1 (6)	1.0 $\pm$ 0.2 (6)	16.7 $\pm$ 2.0 (6)	18.3 $\pm$ 4.0 (6)
2	20	16.6 $\pm$ 2.6 (8)††	17.0 $\pm$ 2.7 (9)††	15.3 $\pm$ 1.6 (8)	14.7 $\pm$ 2.1 (8)
20	2	2.7 $\pm$ 0.3 (9)	8.0 $\pm$ 0.8 (9)*	17.0 $\pm$ 1.1 (9)	14.8 $\pm$ 1.6 (10)
20	20	12.1 $\pm$ 1.6 (10)††	24.7 $\pm$ 3.3 (10)*††	16.2 $\pm$ 2.0 (10)	12.5 $\pm$ 0.6 (9)
44	2	0.7 $\pm$ 0.1 (5)	9.7 $\pm$ 1.5 (5)*	10.8 $\pm$ 2.1 (5)	3.9 $\pm$ 0.5* (5)
44	20	3.0 $\pm$ 0.9 (5)†	9.4 $\pm$ 1.5 (5)*	10.8 $\pm$ 1.4 (5)	4.0 $\pm$ 0.7* (5)

was unaffected (Table 3); this is particularly noteworthy since, at this time, protein synthesis is already inhibited. From 20 h onwards, infected islets hypersecreted insulin. This could be due to acute β -cell damage, or may involve virus-induced alterations of factors that mediate insulin release. Thus EMC infection has been shown to perturb both the balance of intracellular ions [27] and adenylate cyclase activity [8], which are important modulators of insulin release [32]. Furthermore, the cell-to-cell contacts in the islets that are essential for normal levels of insulin release [25] may be decreased as a consequence of infected β -cells becoming distorted and pyknotic [6].

During the 44 h incubation period, the secretory function in response to glucose in the control islets was depressed. This has been observed before, and is typical of cultured islets [12]. Thus, on account of this change in islet function, comparisons were only made between results from control and infected islets obtained at the same time post-infection.

The insulin content of infected islets was not affected until 44 h post-infection (Table 3). Levels were decreased to less than 40% of control values. Such a loss in insulin content is probably a

consequence of the dramatic inhibition of insulin biosynthesis, as well as of elevated rates of insulin release.

To further investigate the regulation of glucose-stimulated insulin biosynthesis by EMC infection, levels of insulin mRNA were measured by nucleic acid hybridization. As a control for RNA recovery, levels of GAPDH mRNA were measured. Preliminary results have been reported elsewhere [33]. Insulin mRNA was depressed as early as 12 h after virus infection, whereas GAPDH mRNA was not significantly affected until 44 h (Fig. 2). Both mRNAs were decreased to less than 20% of control values at 44 h. The dramatic loss of both mRNAs, and possibly other key mRNAs which code for enzymes involved in fuel metabolism, may be responsible for the observed virus-mediated inhibition of glucose-stimulated insulin biosynthesis.

How EMC virus perturbs mRNA levels in infected islet cells has not yet been characterized. This process might involve either an inhibition of transcription [26] or enhanced mRNA degradation [2]. In support of the latter, it is known that the M strain of this virus, as used in this study, contains two distinct variants; the B variant, which is 'non-diabetogenic', and the D variant,

Fig. 2. Insulin mRNA and GAPDH mRNA in EMC-infected islets

Freshly isolated islets were infected with EMC virus at 10^4 p.f.u./islet (see the text). Control (●) and infected (○) islets were then cultured in RPMI 1640 (10% FCS) for 12, 20, 33 and 44 h. Insulin mRNA (a) and GAPDH mRNA (b) were then measured by dot-blot analysis (see the text). Results represent the means for at least four individual determinations, with S.E.M. values shown by bars. Results where $P < 0.01$ for differences between experimental and equivalent controls are denoted by *, and where $P < 0.001$ by **.

which is 'diabetogenic' [34]. The B variant causes slight β -cell damage and induces substantial amounts of interferon which inhibits the lytic action of the D variant [34]. Thus it is most probable that the EMC(M) virus is inducing interferon in infected islets. Interferon has been shown to inhibit insulin biosynthesis selectively [35]. Furthermore, interferon induces latent 2'-5'-oligoadenylate-dependent ribonuclease activity in islets [36]. In the presence of 2'-5'-oligoadenylates, this ribonuclease cleaves mRNA, rRNA and single-stranded viral RNA [37]. In other systems interferon treatment has been shown to lead to decreases in the levels of different mRNAs at dissimilar rates [38], with GAPDH mRNA remaining stable for up to 24 h [39]. Thus the early disparity between the levels of insulin mRNA and GAPDH mRNA, and the later dramatic decrease in both mRNAs (Fig. 2), may be due to the combined effects of interferon and virus infection [34].

EMC virus replication was also shown to occur in the islets. Notably, after an initial low level of infection, virus replication was sustained throughout the period of investigation (T. Ward, M. J. Clemens & K. W. Taylor, unpublished work). At 2 mM-glucose the incorporation of [³H]leucine into total protein in the infected islets at 44 h was only lowered by 25% relative to the uninfected controls. Therefore substantial residual metabolic function was clearly evident within the infected islets. At 44 h there is a selective loss of tissue function rather than gross cytolysis. The survival rate of control and infected islets was also assessed by counting the number of islets recovered from tissue culture after 44 h; 94% of control islets and 78% of infected islets were recovered. The loss of islets in the infected groups was therefore small, and cannot explain the results obtained. Moreover, in the mRNA experiments, only recovered islets were used.

In conclusion, this study shows for the first time that, in islets, EMC virus infection induces an early inhibition of insulin biosynthesis, which is followed by a dramatic decrease in insulin mRNA, accompanied by a selective decrease in insulin biosynthesis. The early disparity in the levels of insulin mRNA and GAPDH mRNA suggests differential effects on the transcription or turnover of these mRNAs. The involvement of interferons in this process, and their role in virus-induced inhibition of protein synthesis in islets, merits further investigation.

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