Isolated Mouse Pancreatic Islets in Culture: Effects of Serum and Different Culture Media on the Insulin Production of the Islets

A. Andersson

Department of Histology, University of Uppsala, Uppsala, Sweden

Summary. Various conditions for tissue culture of collagenase-isolated mouse pancreatic islets were studied in an attempt to optimize the maintenance of glucose stimulated insulin biosynthesis and release in the cultured specimens. Islets which had been cultured at a physiological glucose concentration (5.5 mmol/l) in the absence of serum had an impaired glucose-stimulated insulin biosynthesis and release as well as a reduced insulin content. Thus, insulin biosynthesis was three times higher after culture in a serum supplemented medium. Further, the insulin secretion of islets cultured in the presence of serum was markedly enhanced in acute incubations with high concentrations of glucose. This response was most pronounced in islets which had been cultured free-floating. A comparison between different culture media showed that islets cultured in RPMI 1640 had the highest insulin production. The present data suggest that the most favourable conditions for long-term storage of isolated islets in culture may be obtained when the islets are maintained as free-floating explants in a culture medium consisting of RPMI 1640 supplemented with serum.

Key words: Pancreatic islets, tissue culture, calf serum, culture media, insulin secretion, insulin content, insulin biosynthesis.

Attempts to cure diabetes by pancreatic transplantation have so far met with very little success. On the other hand numerous studies have shown that transplantation of isolated islets into animals with induced diabetes leads to control of plasma glucose as well as a regression of secondary lesions of the disease (for a review see ref. 22). Since islet yield after isolation from the human pancreas is very low, some kind of a tissue bank making possible the successive collection of human islets from several donors will be needed in order to obtain a sufficient number of islets to treat human diabetes. Successful attempts to maintain human islets, isolated from both fetal [14] and adult donors [5] in culture have recently been published.

There is, however, some controversy as regards the optimal conditions for the maintenance of competent islets in culture. Serum seems to favour the maintenance of a normal insulin response to an acute glucose stimulus [3], although it has been possible to keep islets functional in vitro according to the above criterion for prolonged time periods in the absence of serum, provided that the glucose concentration of the culture medium was markedly elevated [9, 13]. Serum supplementation of the medium results in attachment of the islets and a tendency for monolayer formation [4], which may introduce functional disturbances after prolonged culture periods [14] and complicates the harvest of the cultured islets. In the present study a comparative examination has been carried out on islets cultured either attached to the bottom of the Petri dishes or free-floating, in the presence or absence of calf serum. Furthermore a comparative study has been made of the capacity of different commercially available tissue culture media to maintain the insulin production of the cultured islets at a maximal level.

Materials and Methods

Chemicals. Collagenase was obtained from Worthington Biochemical Corp., Freehold, N. J., U. S. A. Tissue culture medium (TCM 199, either glucose-free or containing 5.5 mmol/l D-glucose), Hanks' solution and calf serum were supplied by Statens Bakteriologiska Laboratorium, Stockholm, Sweden, whereas all other culture media were from Flow Laboratories Ltd, Irvine, Ayrshire,

Table 1. Recovery of intact islets after culture in the presence or absence of serum

Day of culture	NUNC + serum	HEGER + serum	NUNC − serum
0	50 (10)	50 (11)	50 (12)
3	40±3 (10)	35±2 (11)	34±4 (12)
5	35±4 (10)	30±3 (11)	30±4 (12)
7	35±4 (10)	29±3 (11)	17±3 (12)[a,b]

At the beginning of the culture period fifty islets were suspended in each Petri dish, containing 5 ml TCM 199 with or without 10% (v/v) calf serum. Islets cultured in NUNC-dishes in the presence of serum stuck to the bottom of the dish, which was not the case when the experiments were performed in the absence of serum or in HEGER-dishes. The number of islets (mean ± SE) was estimated on the 3rd and 5th day of culture as well as at the end of the culture period. Significance of the difference between no addition and addition of serum: [a] = $p < 0.005$ versus culture in NUNC-dish; [b] = $p < 0.02$ versus culture in HEGER-dish

Scotland. Penicillin and streptomycin were from Glaxo Laboratories Ltd, Greenford, Middx, U.K. Bovine plasma albumin (fraction V) was obtained from Armour Pharmaceutical Co., Eastbourne, Sussex, U.K. ^{125}I-labelled insulin and L- [4,5-^{3}H]leucine (53 or 119 Ci/mmol) were supplied by the Radiochemical Centre, Amersham, U.K. Antiserum against bovine insulin was from Miles Laboratories Inc., Kankakee, Ill., U.S.A. and crystalline mouse insulin from Novo A/S, Copenhagen, Denmark. CNBr-activated Sepharose 4B was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden and Soluene from Packard Instrument Co., Downers Grove, Ill., U.S.A. Other chemicals used were of analytical grade.

Preparation and Culture of Isolated Islets. Pancreatic islets, obtained by a collagenase method [17] were obtained from adult NMRI-mice (Anticimex, Sollentuna, Stockholm, Sweden), which were starved over-night before the experiments. After isolation the islets were immediately transferred by means of a braking pipette to plastic Petri dishes containing 5 ml tissue culture medium (TCM 199) supplemented with penicillin 100 U/ml and streptomycin 0.1 mg/ml. The following three culture conditions were used: 1) Culture in a medium supplemented with 10% (v/v) calf serum in Petri dishes especially designed for tissue culture (NUNC, Roskilde, Denmark); 2) Culture in the same dishes but in the absence of serum; 3) Culture in dishes made of ordinary plastic (HEGER Plastics, Oslo, Norway), which do not permit attachment of the islets, despite the presence of 10% (v/v) calf serum.

The glucose concentration of the culture medium was 5.5 mmol/l or as given below and the dishes were incubated at 37°C in a gas phase consisting of 5% CO_2 in humidified air. The medium was changed on the third and fifth days of culture. After 7 days of culture the islets were harvested mechanically by means of a rubber policeman and/or a braking pipette [3]. In some experiments the islets were counted when viewed in a stereomicroscope both when the medium was changed and when the islets were harvested.

Culture experiments to test the effects of different culture media were performed in Linbro multidishes model FB-6-S (Flow Laboratories Ltd, Irvine, Ayrshire, Scotland), in which the islets did not become attached to the bottom of the dish. At each medium change samples of the media were taken for insulin assay. In order to avoid admixture with free-floating islets about 500 µl of the culture medium was sucked into a syringe under microscopic control. The sample was transferred to a test tube and left for

about 5 min before removal of 10–25 µl of the supernatant for insulin assay.

Insulin Secretion and Content. Groups of 5 or 10 cultured islets were incubated for 60 min at 37°C in glass vials [19] containing 250 µl Krebs-Ringer bicarbonate buffer [20] supplemented with 10 mmol/l HEPES, 2 mg/ml of bovine albumin and 1.5 or 15 mmol/l glucose. Before the experiment each vial was gassed for 1 min with $O_2 + CO_2$ (95:5). At the end of the incubation period the medium was removed, frozen and stored at −20°C prior to insulin assay.

For the measurements of the islet insulin content groups of 10 cultured islets were washed briefly in Hanks' solution and homogenized by sonication for 30 s in 500 µl acid ethanol (15 ml 12 mol/l HCl in 70% ethanol) and extracted over-night at 4°C. The extracts were stored at −20°C before the insulin assay, which was performed by a radioimmunoassay procedure [15] using crystalline mouse insulin as a standard.

Insulin Biosynthesis. After the islets had been recovered from the culture dishes groups of 10 islets were incubated in 100 µCi/ml L-[4,5-^{3}H]leucine and 20 µg/ml of each of 12 naturally occurring amino acids as according to Eagle [11] except that non-radioactive L-leucine was omitted. Incubations were performed for 2 h at 37°C in a gas phase consisting of 5% CO_2:95% O_2. At the end of the incubation period the islets were washed and homogenized by sonication. The homogenate was then subjected to a modified immuno-binding technique [8] by incubation for 2 h in phosphate buffered saline containing excess antibovine insulin antiserum coupled to Sepharose 4B beads. Control values obtained by incubation of samples of the same homogenate with normal guinea-pig serum proteins coupled to Sepharose beads were subtracted from the experimental values. The incorporation of [^{3}H]-leucine into the total protein pool of the cultured islets was estimated by precipitating samples of the islet homogenates with 10% (w/v) trichloroacetic acid (TCA).

Results

Effects of Serum

Recovery of Islets after Culture. As shown in Table 1 there was a decreased number of islets at the end of the 7 day culture period, irrespective of the culture protocol. This reduction in the number of cultured islets was most pronounced for the islets cultured in the absence of serum in which case only one third of the originally explanted islets could be recovered at the end of the culture period. No significant difference was found between islets cultured in the presence of serum and either free-floating (HEGER) or attached to the bottom of the culture dish (NUNC), although the number of free-floating islets was about 15% lower on all the three occasions estimates were made. In these two groups the loss of islets occurred mainly during the first three days of the culture period.

Insulin Secretion. The ability of glucose (15 mmol/l) to stimulate the insulin release of the cultured islets is shown in Figure 1. The insulin secretion of the islets
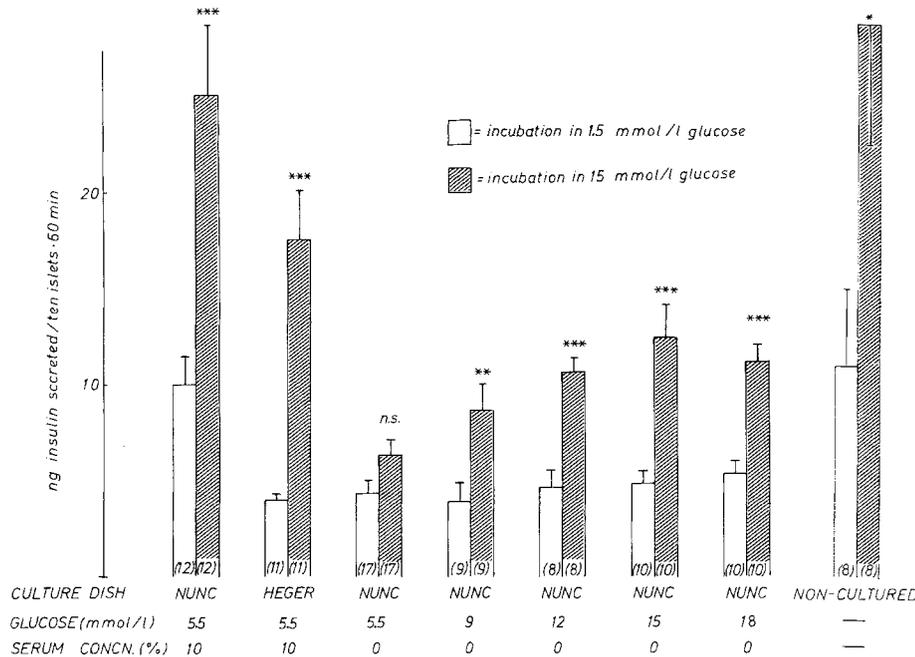
Fig. 1. Islets were cultured for 1 week in TCM 199 either free-floating in the presence of serum (HEGER-dish) or in NUNC-dishes permitting adherence, when serum was present in the culture medium. The insulin release (mean ± SE) was estimated in short-term incubations (60 min) at the end of the culture period either in 1.5 mmol/l glucose (open bars) or in 15 mmol/l glucose (hatched bars). Statistical significance of the difference between low and high concentrations of glucose: $* = p < 0.05$; $** = p < 0.01$; $*** = p < 0.001$; n. s. = non-significant

cultured in TCM 199 at 5.5 mmol/l glucose in the presence of 10% (v/v) calf serum was significantly enhanced irrespective of whether the islets were cultured attached to the bottom of the culture dish (NUNC) or as free-floating explants (HEGER). The more than four-fold increase of insulin release in response to the high extracellular glucose concentration can be attributed to a lower insulin release from the non-attached islets at 1.5 mmol/l glucose, while the corresponding factor for the attached islets was two and a half. These islets displayed a secretory pattern almost identical to that of the non-cultured islets.

The insulin secretion of the islets cultured at 5.5 mmol/l glucose in the absence of serum was not stimulated by 15 mmol/l glucose in the short-term incubation. When the glucose concentration of the culture medium was increased, a small but significant enhancement of the insulin release was obtained. This stimulatory capacity of glucose was most pronounced after culture at the highest glucose concentrations, although it never reached that observed after culture in the presence of serum.

In a separate series of experiments the lack of an effect of 60 min heat inactivation at 56°C of the calf serum was established. Thus, there was no difference observed in the insulin release capacity in short-term experiments of islets cultured free-floating in TCM 199 for one week in 5.5 mmol/l glucose in the presence of normal or heat-inactivated calf serum. The secretion figures (mean values ± SE) recorded for these two particular groups of islets were 1.1 ±

0.2 and 1.5 ± 0.3 ng insulin/5 islets/60 min (n = 10) respectively in 1.5 mmol/l glucose; the corresponding values in 15 mmol/l glucose were 4.3 ± 1.1 and 5.6 ± 1.5 ng insulin/5 islets/60 min (n = 10). Neither was there any difference observed in the insulin content; islets cultured in non-heated serum contained 310 ± 48 ng insulin/10 islets (n = 5), whereas those cultured in heat-inactivated serum contained 383 ± 60 ng insulin/10 islets (n = 5, mean values ± SE).

Insulin Content and Biosynthesis. There was no major difference in the insulin content of the two groups of islets cultured in the presence of calf serum, but both contained only about one third of the insulin found in the non-cultured islets (Table 2). Islets cultured in a serum-free medium at 5.5 mmol/l glucose, however, had an insulin content about 25% below that of the two other groups of cultured islets. Furthermore, these particular islets synthesized insulin at a considerably lower rate, as evidenced by their markedly reduced capacity to incorporate tritiated leucine into the proinsulin-insulin pool at the end of the culture period, in the presence of a high extracellular glucose concentration. Insulin biosynthesis, in percentage of the total protein synthesis, was about three times higher after culture in the presence of calf serum. Nevertheless, this figure for the islets cultured in the presence of serum is only half that observed for the non-cultured islets. This was mainly due to an increase of the rate of total protein synthesis (TCA) in all groups of cultured islets.

Table 2. Insulin biosynthesis and content of islets cultured in the presence or absence of serum

Culture condition (mmol/l glucose)	PI-I (cpm × 10⁻³/2 h per 10 islets)	TCA	%		Insulin content (ng/10 islets)	
NUNC, + serum, 5.5	11.4±1.6	74.1±10.6	12.9±1.6	(12)	308±23	(16)
NUNC, no serum, 5.5	2.7±0.3^c	71.5±, 7.6	4.3±0.4^c	(11)	216±19^b	(14)
HEGER, + serum, 5.5	8.9±1.0	77.6± 8.9	11.5±1.8	(9)	325±25	(12)
NUNC, + serum, 18	10.9±1.0	62.9±10.6	17.6±1.7^d	(8)	92±19^e	(6)
NUNC, no serum, 18	8.1±1.0^e	62.0± 6.5	12.8±1.0^{e,a}	(7)	54±13^e	(5)
HEGER, + serum, 18	11.5±0.7^d	70.5± 9.6	16.1±1.6^d	(5)	120±10^e	(3)
Non-cultured	14.0±1.9	31.6± 3.6	26.4±0.9	(8)	1107±130	(8)

Islets were cultured for one week in NUNC-dishes (permittance of adherence) or HEGER-dishes (no attachment) containing 5 ml TCM 199 with or without 10% (v/v) calf serum and glucose at a concentration of 5.5 or 18 mmol/l. Insulin biosynthesis was estimated by measuring the incorporation of tritiated leucine (53 Ci/mmol) at 16.7 mmol/l glucose into both the (pro)insulin fraction (PI-I) and TCA-precipitable protein fraction (TCA) of the islet homogenate. The values in column (%) refer to the percentage of the total incorporated radioactivity represented by the PI-I fraction as calculated from each individual observation. Insulin was estimated by radioimmunoassay of acid alcoholic extracts of homogenates of 10 islets. Means ± SE are given as well as the statistical significance of the difference between culture in NUNC-dishes in the absence or presence of serum (^{a} = $p < 0.05$; ^{b} = $p < 0.01$; ^{c} = $p < 0.001$) or between culture at 5.5 or 18 mmol/l glucose (^{d} = $p < 0.05$; ^{e} = $p < 0.001$). Numbers of experiments are given within parentheses

Table 3. Summary of the composition of the culture media tested

Constituents	TCM 199		RPMI 1640		CMRL 1066		MEM-Eagle		HAM's F10	
Inorganic salts	9198	(8)	9349	(6)	9940	(6)	7896	(7)	9231	(10)
Ca^{2+}	1.26		0.43		1.80		1.26		0.30	
PO$_4$$^{3-}$	0.77		4.50		1.00		0.77		1.69	
Amino acids	1100	(21)	1009	(20)	1020	(21)	852	(13)	553	(20)
L-arginine	0.33		1.14		0.40		0.60		1.00	
L-leucine	0.46		0.40		0.46		0.40		0.10	
Vitamins	1.0	(16)	43.7	(11)	51.0	(13)	8.1	(8)	6.9	(10)
ascorbic acid	0.0003		—		0.305		—		--	
i-inositol	0.0003		0.194		0.0003		0.011		0.003	
nicotinamide	0.0002		0.0082		0.0002		0.0082		0.005	
Other components	1104	(16)	2006	(3)	1182	(19)	1010	(2)	1216	(6)
glucose	5.5		11		5.5		5.5		6.1	
Total number of constituents	61		40		59		30		46	

The composition of the five different commercially available tissue culture media used in the present study. The listing has been confined to four major groups of constituents given as mg/l with the number of single components in each group within parentheses. The concentrations of some substances of special interest have been given in terms of molarity (mmol/l). Information was obtained from a survey published by Morton [23] on behalf of the Standards Committee of the Tissue Culture Association

By increasing the glucose concentration of the culture medium to 18 mmol/l a further decrease of the insulin content of the cultured islets was obtained. Once again the serum supplementation was of less benefit at this high glucose concentration and the only significant difference occurred in the percentage figure for insulin biosynthesis, which was lower for the islets cultured in the absence of serum. The islets cultured in the presence of serum responded to the higher glucose concentration of the culture medium with an increase of the percentage figure, whereas only small differences were found in the rate of incorporation of tritiated leucine into the proinsulin-insulin pool.

Effects of Different Culture Media

Insulin Accumulation in the Culture Medium. The composition of the different culture media is given in condensed form in Table 3. As can be seen in Figure 2 there was a gradual decrease of the insulin accumulation in the culture medium from the third to seventh day of culture for all culture media tested. The highest values were recorded for the media containing 11 mmol/l glucose, of which RPMI 1640 was found to be the most effective. Thus, there was a four-fold enhancement of the insulin accumulation in TCM 199 when the glucose concentration was increased from 5.5 to 11 mmol/l ($p < 0.001$ for
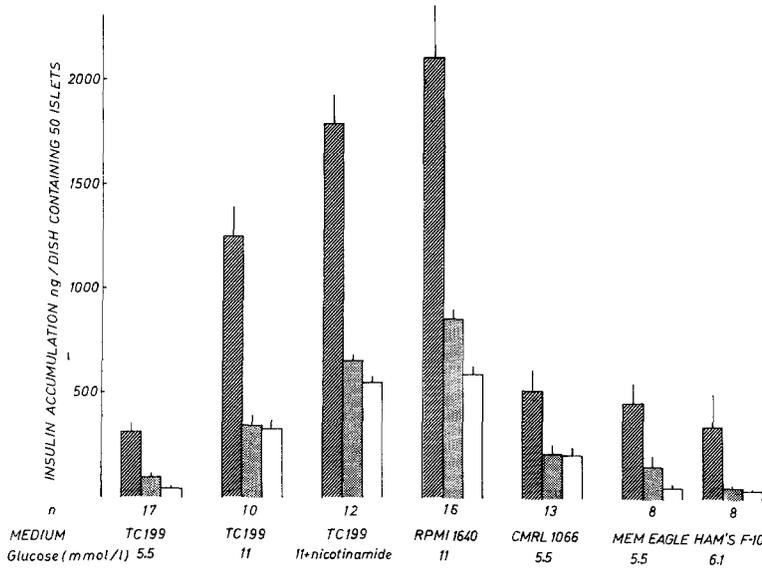
Fig. 2. Accumulation of immunoreactive insulin (mean ± SE) in different culture media. Nicotinamide 8.2 μmol/l was added to TCM 199 with 11 mmol/l glucose. For a further description of the composition of the different media, see table 3. The medium was changed after 3 (hatched bars) or 5 (dotted bars) days culture and a sample of the medium was also taken at the end of the culture period (7th day — open bars)
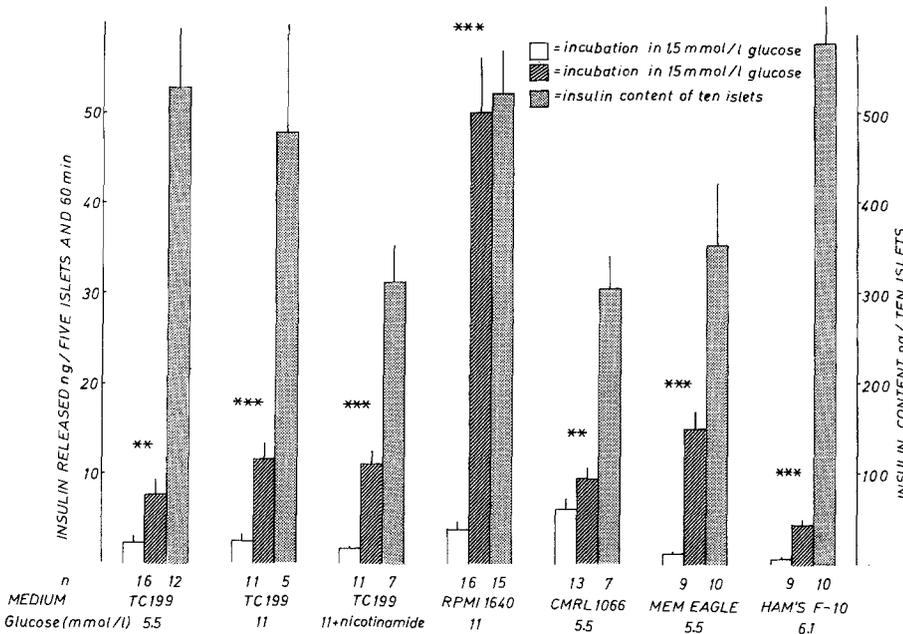

Fig. 3. Islets were cultured free-floating for 1 week in different culture media and in the presence of 10% (v/v) calf serum. The concentration of nicotinamide was 8.2 μmol/l. The insulin release (mean ± SE) was estimated in short-term incubations either in 1.5 mmol/l glucose (open bars) or in 15 mmol/l glucose (hatched bars), the statistical significance of the difference between low and high concentrations of glucose being given at the top of the latter bars. Dotted bars indicate insulin content of the islets at the end of culture period (mean ± SE)

each day tested). Addition of nicotinamide, 0.0082 mmol/l, to TCM 199 with 11 mmol/l glucose further stimulated insulin secretion (p < 0.01; p < 0.001 days 5 and 7). It should be noted, however, that corrections of the insulin values have been made neither for the degradation of insulin in the culture medium, nor for the decrease in number of islets during the culture period.

Insulin Content and Secretion. The highest insulin content was found in the islets cultured in HAM's F-10 and the lowest in islets cultured in CMRL 1066 (Fig. 3). After addition of nicotinamide, 0.0082 mmol/l, and glucose up to 11 mmol/l to TCM

199 there was a decrease in the insulin content (p < 0.05); this medium also gave a lower insulin content in comparison with that found after culture in RPMI 1640 (p < 0.01).

At the end of the culture period in the short-term experiments on the insulin response to an acute glucose load there was a significant stimulation for all groups of islets (Fig. 3). The highest absolute values were recorded for islets cultured in RPMI 1640, the lowest being obtained with HAM's F-10 medium. RPMI 1640 also gave the highest stimulation factor, 13.5, i.e. the maximal secretion divided by the basal value. Culture in medium MEM Eagle resulted in a similarly high degree of glucose stimulated insulin

Table 4. Insulin biosynthesis of islets cultured in different media

Culture medium (mmol/l glucose)		PI-I (cpm × 10^{-3}/2 h per 10 islets)	TCA	%
TCM 199,	5.5	9.6±1.8	49.0± 7.4	19.3±1.0
TCM 199,	11.0	11.3±2.3	76.5±17.4	15.4±2.2
RPMI 1640,	11.0	19.3±2.1	78.2±13.3	26.3±2.0
MEM Eagle,	5.5	7.9±2.1	64.4±16.2	12.1±1.3
HAM's F10	6.1	10.5±2.5	70.0±19.3	17.1±1.7
CMRL 1066,	5.5	9.6±2.7	56.9±12.5	16.2±1.5

Islets were cultured free-floating for one week in different culture media and in the presence of 10% calf serum. Insulin biosynthesis was estimated as in Table 2 but the specific radioactivity of the tritiated leucine used was 119 Ci/mol. Means ± SE of six experiments

secretion, whereas CMRL 1066 and TCM 199 with 5.5 mmol/l glucose yielded considerably lower figures, 1.6 and 3.5 respectively.

Insulin Biosynthesis. The rates of insulin biosynthesis at 16.7 mmol/l glucose of the islets cultured in the different culture media are given in Table 4. Again, the highest values were recorded for islets cultured in RPMI 1640 and this was the case both for incorporation of radioactivity into the proinsulin-insulin pool (p < 0.05 vs. each of the other media) and for the percentage figure (p < 0.01). No major differences were observed between the other media tested; islets cultured in MEM Eagle displayed the lowest values. It may be noted, too, that addition of glucose to TCM 199 up to 11 mmol/l was not sufficient to reach the figures recorded for RPMI 1640. Furthermore, the different groups of cultured islets displayed similar rates of total protein synthesis (TCA).

Discussion

Several different techniques for the preparation of isolated islet cultures have been reported (for a review see ref. 3). Clearly such a technique must permit the recovery of the islets at the end of the culture period by means of a non-traumatic harvesting procedure in order to become useful for more detailed functional studies or transplantation purposes. The method developed and used in our laboratory during the last few years [1, 3, 4] has provided both a satisfactory survival of the explanted islets and allowed a careful metabolic characterization and functional study of the cultured islets. The present study aimed at a further definition of the best conditions for tissue culture of collagenase isolated pancreatic islets, when explanted *in vitro* into open plastic Petri dishes.

The data obtained confirmed those of previous studies that the presence of serum during culture at 5.5 mmol/l glucose is necessary for a good survival and a normal insulin response to glucose [3, 21]. However, despite the presence of serum there was a reduction of the number of islets during the first week of culture. The reasons for this include difficulties in performing a complete medium change without simultaneous removal of islets and spontaneous disruption of islets in culture. The first problem is greater in the "free-floating" system. However islet loss also occurs with the other culture method at the first medium change, when some islets have not yet stuck to the bottom of the culture dish. Furthermore, the attachment causes monolayer formation after a few weeks in culture, which makes harvest of the cultured islets somewhat complicated. As regards disruption of islets, this process takes place mainly during the first days of culture. It might well be that the islets lost in this way are injured by the collagenase so that they cannot survive *in vitro*. If so, the enrichment of viable islets is of considerable advantage in that the islet population is more homogeneous at the end of the culture period.

Up to now it has not been clear whether this serum effect arises from chemical factors in the serum or whether it is due to the adherence of islet cells to the bottom of the dish. The present as well as previously published data [9, 13, 27] indicate that isolated pancreatic islets are not anchorage-dependent as regards maintanance of their specific functions during the culture. It seems, on the other hand, as if a higher ratio between the basal and glucose-stimulated insulin release is obtained when the islets are cultured in a serum supplemented medium as free-floating explants instead of attached to the bottom of the Petri dish. Furthermore, such a method for culture of isolated islets is compatible with a high rate of insulin biosynthesis. There are, however, no data yet available about the importance of islet cell attachment for their replication *in vitro* [2, 10] but it may well be that this process is anchorage-dependent like that which has been observed for most other non-neoplastic cells.

The present data also are in agreement with those published by Buitrago *et al.* [9] that a glucose sensitive insulin release can be demonstrated after culture in the absence of serum. A prerequisite, however, was that the glucose concentration of the culture medium was elevated, suggesting that high glucose may substitute for some factor(s) in serum, which promotes preservation of glucose regulated insulin release and biosynthesis of islets in culture. One such factor might possibly be insulin itself, since this hormone has been found to be of primary importance for the survival in culture of explants from the mouse

[12] and to be a serum factor controlling the DNA synthesis *in vitro* of different cell-lines [16, 25].

The present work thus implies that the free-floating culture form in the presence of serum provides the best conditions for storage of collagenase isolated islets for extended periods of time in a tissue bank intended for transplantation purposes. In support of this we have cultured mouse islets in this way for eight months (Höiriis-Nielsen, Brunstedt, Andersson and Frimodt-Möller, to be published) and others have cultured human fetal islets for two to five months [14], the islets remaining morphologically and functionally intact. Furthermore, recent studies in our laboratory have shown that it is possible to maintain pancreatic islets isolated from adult human donors viable under these conditions with an intact biphasic insulin release in response to an acute glucose load [6], the longest experiments having been extended for up to four months (Höiriis-Nielsen, Brunstedt, Andersson and Frimodt-Möller, to be published).

In order to optimize further the conditions for storage of isolated islets in culture studies were also made on the influence of different culture media and heat inactivation of the calf serum on the subsequent insulin release from the cultured islets. Although the latter process has repeatedly been found to decrease the degradation of accumulated insulin in the culture medium [18, 24] and to abolish the functional damage to isolated islet cells induced by human serum [26] we found in this study no beneficial effect of the heat inactivated serum on glucose stimulated insulin response of the cultured islets in the short-term experiments at the end of the culture period. On the other hand very marked differences were observed in the effects of the different culture media examined. The superiority of RPMI 1640 in stimulating insulin production during the culture period and its ability to maintain and even to enhance glucose stimulated insulin secretion cannot be ascribed only to its high content of glucose (11 mmol/l) since adjustment of the glucose concentration of TCM 199 to the same level was less effective. Another difference between the media RPMI 1640 and TCM 199 is the high vitamin content of RPMI 1640 and especially that of nicotinamide, whose concentration is forty times higher. Indeed, the addition of this precursor for important coenzymes to TCM 199, with 11 mmol/l glucose, increased insulin secretion into the culture medium so that only small differences were observed between this particular medium and RPMI 1640. The fact that culture in the supplemented TCM 199 medium gave a lower insulin release response to an acute glucose load as well as a lower islet insulin content, however, suggests that other factors than the

glucose and nicotinamide concentrations are responsible for the different suitability of these two media. It is of interest too, that the superiority of RPMI 1640 is not confined to its effects on the function of the islet B-cells, since similar beneficial effects have also been observed concerning glucagon production of islets in culture (Andersson and Östenson, to be published).

In conclusion this and other studies [14, 21] have demonstrated the importance of the composition of the culture medium for maintaining the specific functions of islet tissue in culture. It may be especially noted that the glucose and leucine concentrations in the culture medium markedly influence the structure and metabolism of islet cells in culture [1, 7], which will certainly be of great importance both for islet storage and islet function before transplantation to diabetic recipients.

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Dr. A. Andersson
Department of Histology
University of Uppsala
Box 571
S-751 23 Uppsala, Sweden