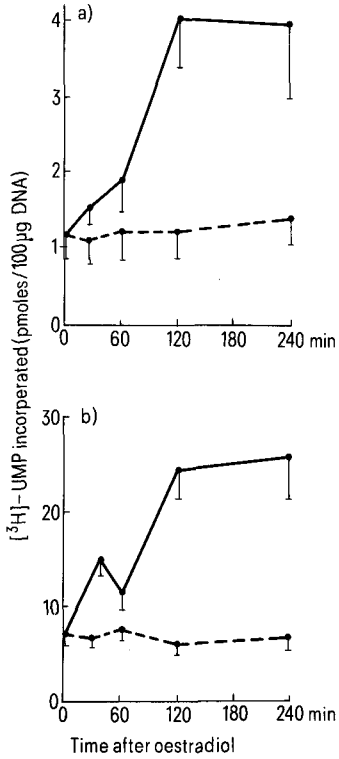
30 min; there was a 4-fold increase at 120 min. The level remained constant till 240 min. In contrast, in the foetal kidney, after 120 min of oestradiol treatment, no increase was observed. The control values for RNA polymerase activities I and II were 0.7 pmoles/100 µg DNA ± 0.24 (SEM) and 3.8 ± 0.6 (SEM) respectively, and in oestradiol treated foetuses were 0.8 ± 0.6 (SEM) and 4 ± 0.36 (SEM).



Effect of oestradiol treatment on RNA polymerase I and II activities in the uterine nuclei of foetal guinea-pig. Foetuses were removed at different times up to 240 min after a single injection of oestradiol as described in the text. Uterine nuclei were prepared and endogenous RNA polymerase activities determined. *a* RNA polymerase I activity: oestrogen treated (—) and control (- - -). *b* RNA polymerase II activity: oestrogen treated (—) and control (- - -) uteri. All points represent the mean ± SEM from 3-4 determinations.

This is an additional effect to the different oestrogenic responses already observed in the foetal uterus and, to our knowledge, has not been described in the foetus. It is interesting to note that the increase in RNA polymerase activities above control values within the 1st 240 min of treatment is similar to that of immature or ovariectomized rabbit and rat[8,11].

Since the foetal uterus of the guinea-pig responds to oestradiol by a very rapid increase in histone acetylation[4] (after 10 min of treatment), it is possible that this enhancement of histone acetylation might be the first step before the increase in RNA polymerase activities. The latter might precede the incorporation of ^{3}H-leucine into acid-insoluble proteins previously observed[5]. Moreover, it has been demonstrated that oestradiol treatment provokes the induction of a specific protein, namely the progesterone receptor[2,12]. Consequently, it can be suggested that in this foetal tissue there exists a correlation between the activation of RNA polymerases by oestradiol and the synthesis of proteins.

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Pancreatic islet cell suspensions from newborn rats; different preparation procedures, viability and (pro)insulin biosynthesis[1]

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Summary. Islet cell suspensions were prepared from neonatal rat pancreatic islets. While mechanical disintegration results in a higher yield, cells prepared by trypsin treatment appear to be better preserved. Trypsin treatment of pancreatic islets during the cell preparation procedure does not influence the stimulation by glucose of (pro)insulin biosynthesis in freshly isolated cells.

The isolation of pancreatic islet cells of different species[2-8] has become an important tool for the investigation of special problems in experimental diabetes research.

Cell suspensions have been prepared from isolated islets of Langerhans by mechanical disintegration[4], enzymatic treatment[2,3,6,9] or a combination of both methods[5]. We compared 2 of these procedures with respect to the yield and the stability of the cells. Because the glucose stimulation of (pro)insulin biosynthesis of cells prepared by mechanical disintegration has already been shown[10,11], the question

Comparison of some characteristics of cell suspensions freshly prepared from islets of Langerhans of neonatal rats by mechanical disintegration or trypsin treatment

	Method of cell preparation Trypsin treatment	n	Mechanical disintegration	n
Number of cells isolated per 100 islets	$2\times10^4\pm2{,}4\times10^3$	11	$7.1\times10^4\pm8.1\times10^{3**}$	6
Percent trypan blue positive cells	1.4 ± 0.16	10	$19\pm1.36^{**}$	14
Percent ^{51}Cr-release	2.74 ± 0.30	4	$8.75\pm2.09^{*}$	5

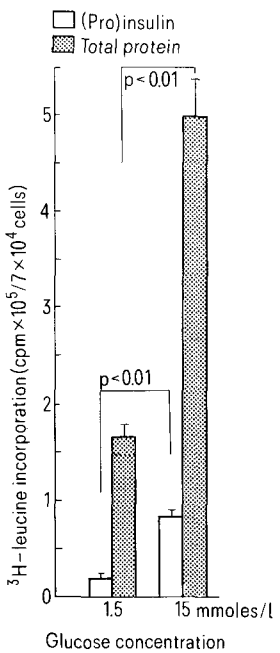
Mean ± SEM $^{**}p<0.01$; $^{*}p<0.05$

arises as to whether the trypsin treatment of the islets of Langerhans affects the glucose stimulation of (pro)insulin biosynthesis of the isolated cells.

Material and methods. Neonatal Wistar rats 4–12 days old were used throughout the study. Pancreatic islets fom 15 to 20 pancreata were isolated by a modified fractionated collagenase (SERVA GmbH, Heidelberg (FRG), 0.3 Mandl units/mg) digestion[12]. The pancreas were digested 3 times with 5 ml Hank's solution containing 1 mg collagenase/ml followed by Ficoll density-gradient centrifugation[13]. Then the islets were collected under a stereomicroscope. In the first procedure, the preparation of islet cell suspensions was done using the mechanical disintegration method of Lernmark[4]. The isolated islets were transfered into 200 µl of a Ca^{2+}-free Hank's solution supplemented with 3 mmoles/l EDTA, 1.5 mmoles/l glucose and 0.5% bovine serum albumin (BSA, Behring Werke, Marburg, FRG) and immediately were shaken vigorously (Whirlmixer, Fisons Scientific Apparatus, England) for 15–20 sec.

In the other procedure, freshly isolated islets were transfered into 1 ml of Ca^{2+}-free Hank's solution as stated above, and preincubated for 30 min at room temperature. The islets were then disrupted into single cells by trypsin treatment in a Ca^{2+}-free Hank's solution containing 0.01% trypsin, 0.1% bovine serum albumin and 1.5 mmoles/l glucose. Depending on the assay which was to follow, the cells were washed by centrifugation (5 min at $50\times g$) with TCM 199 containing 10% fetal calf serum, with Hank's solution, or with the medium used for studies of (pro)insulin biosynthesis supplemented with 1% bovine serum albumin. The trypan blue exclusion test[14] was used to evaluate the viability of the cells. In addition, spontaneous ^{51}Chromium release[5] after 15 min incubation from islet cells prelabeled with $Na_2{}^{51}CrO_4$ for 60 min was also determined and taken as a measure of damage to the cells.

(Pro)insulin biosynthesis in freshly prepared islet cell suspensions was determined in nearly the same way as was previously described in detail for isolated islets of Langerhans[15]. In brief, a defined number of islet cells was incubated in a shaking water bath for 2 h at 37 °C in the presence of 10 µCi of ^{3}H-leucine (Amersham, sp.act. 40–60 Ci/mmole) in 200 µl Krebs-Ringer bicarbonate buffer supplemented with 19 naturally occurring amino acids, leucine excluded, and 1.5 mmoles/l or 15 mmoles/l glucose. Before use, the medium was gassed with 5% CO_2/95% O_2 at pH 7.4. At the end of the incubation period the cells were destroyed by freezing in a mixture of dry ice/acetone and thawing in a water bath several times, and the protein was precipitated by trichloroacetic acid (TCA, final concentration 6.6% w/v). After dissolving the TCA-precipitate in 100 µl acetic acid, an aliquot was used to determine total protein synthesis. Newly synthesized (pro)insulin was determined immunologically by a modification of the double-antibody method[16].



Incorporation of ^{3}H-leucine into (pro)insulin as well as total protein at different glucose concentrations during a 2-h incubation of islet cell suspensions freshly prepared by trypsin digestion from islets of Langerhans of neonatal rats. Mean ± SEM n = 3, $p<0.01$.

Results and discussion. In our study a comparison of the mechanical and the enzymatic methods used to prepare dispersed cell suspensions of pancreatic islets from neonatal rats showed that mechanical disintegration of the islets results in a higher yield of cells. On average 7.1×10^4 cells were isolated per 100 islets of newborn rats (table). This is comparable with the yield obtained by the same method from adult rats[17] but is lower than yields reported for adult ob/ob mice (2.2×10^6 cells per 200–500 islets)[4]. In contrast, the preparation of islet cell suspensions by trypsin treatment is associated with a considerable loss of cells: an average yield is only 2×10^4 cells per 100 islets (table). After trypsin treatment of islets from adult rats, Pipeleers et al.[18] isolated approximately 5×10^4 cells per 100 islets. This higher yield may be due to differences between the islets of adult and newborn rats as well as some differences in the preparation procedure[18]. With respect to the preservation of the isolated cells the trypsin treatment is better than the mechanical disintegration. This result was confirmed by a higher number of trypan blue positive cells as well as a higher percentage of ^{51}Cr-release from prelabeled islet cells prepared by the mechanical method (table).

Age differences in the animals do not seem to bring striking differences in the sensitivity of rat islet cells to trypsin treatment (0,01%); the percentage of trypan blue positive cells in pancreatic islet cell suspensions of newborn (1.4%, table) and adult rats (< 4%)[18] were comparable. The relatively high portion of trypan blue positive cells (19%) observed after mechanical disintegration of the islets does not seem to be specific for the preparation of islet cells of newborn rats because comparable results were also reported for isolated pancreatic islet cells from ob/ob mice[4] and

adult rats[17]. The trypsin concentration (0.01%) sufficient to prepare islet cells from newborn rats as well as adult rats[18] was not suitable for preparing pancreatic islet cell suspensions from C57BL/6J mice (results not shown). The choice of a particular trypsin concentration and the digestion period which permits the isolation of islet cells in a high yield but with a small degree of damage of the cells depends on the species and the age of the animals used.

Lernmark[10] and Niki et al.[11] have already shown that pancreatic islet cell suspensions of adult rats prepared by mechanical disintegration respond normally to glucose stimulation with an increased (pro)insulin biosynthesis. Therefore it was of interest to investigate whether the trypsin treatment of the pancreatic islets of newborn rats has an influence on the ability of the isolated cells to synthesize (pro)insulin.

We found that cells isolated by trypsin treatment of islets of Langerhans of newborn rats also responded with a significantly increased (pro)insulin biosynthesis to glucose stimulation (fig.). In a series of experiments with different cell numbers per tube, we observed a 4–8-fold stimulation of (pro)insulin biosynthesis when glucose was increased from 1.5 mmoles/l to 15 mmoles/l. A number of 1×10^4 cells per tube was already sufficient to detect the glucose stimulation of (pro)insulin biosynthesis (data not shown). These results clearly demonstrate that the glucose recognition mechanism as a necessary prerequisite for the stimulation of (pro)insulin biosynthesis is not altered by the enzymatic treatment of the cells during the preparation procedure. A portion of $19.8\% \pm 1.58$ ($n=7$) of the total protein synthesized in a 2 h incubation at a glucose concentration of 15 mmoles/l was determined to be (pro)insulin. In comparison the proportion of (pro)insulin synthesized by intact islets of Langerhans of newborn rats amounted to only $11.6\% \pm 0.60$ ($n=9$) of the total protein. This suggests a certain enrichment of β-cells in the islet cell suspension, possibly due to a removal of non-insulin producing cells during the preparation procedure.

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Effect of estrogen administration on the induction of the plasma prolactin afternoon surge and on anterior pituitary prolactin concentration extracted at different pHs[1]

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Summary. Ovariectomized (OVX) rats were injected with various doses of polyestradiol phosphate (PEP); the anterior pituitary (AP) prolactin (PRL) concentration and the plasma afternoon surge of PRL were observed 1 week later by radioimmunoassay. AP PRL was extracted using carbonate and phosphate buffers at either pH 7.6 or 10.6. The AP concentration of PRL was greater when the AP was extracted with buffers at pH 10.6 and the phosphate buffer was the most efficient. The concentration of PRL in the AP more closely reflected the magnitude of the estrogen-induced afternoon surge when the AP was extracted at pH 10.6 and this was especially so when the higher levels of estrogen were administered.

Before the advent of RIA the effect of estrogen on prolactin (PRL) secretion could only be determined by measuring the anterior pituitary (AP) content[4–6]. Once specific RIAs were firmly established to measure plasma PRL levels, the AP was usually overlooked in examining the influence of estrogen on prolactin secretion[7,8]. In some studies where AP and plasma PRL were measured, the correlation between the two was not always good[9]. Part of the reason for this poor correlation may have been due to the method of extracting PRL from the AP for RIA[10,11], while another part may have been due to making comparisons to only basal plasma levels[9]. In this study we examined the influence of various doses of polyestradiol phosphate (PEP), a long acting estradiol, on the induction of the afternoon plasma PRL surge and compared this response with the AP PRL concentration extracted with different buffers at various pHs. It was the intent of this study to put into proper perspective the relationship between AP PRL concentration and plasma levels when stimulated by estrogen.