were pooled together and applied to the column. As shown in figure 3, there were no differences in the elution profiles of control and CPH-treated islet extracts.

Discussion. It has been suggested that the diabetogenic action of CPH in rats is due to a specific action on the beta cells of pancreas. The compound causes histologic abnormalities exclusively in beta cells, insulin depletion of pancreas or pancreatic islets and inhibition of insulin secretion. These observations strongly sugget that CPH may impair the insulin biosynthetic process[11].

On the contrary, the present study demonstrated that the insulin biosynthesis of CPH-treated rats is almost normal with respect to rate and amount. The mechanism by which CPH causes marked and rapid depletion of insulin content of pancreatic islets is, however, not clear since the compound is not reported to stimulate insulin secretion from islets. One explanation could be the leakage of insulin. Though CPH has been reported to inhibit insulin release from isolated islets in vitro, the high concentration of CPH in the medium (1 mM), in contrast, is able to induce a large release of insulin from isolated islets probably by causing beta cell damage[14]. The dosage of CPH used in our study and others[3,4], 45 mg/kg, i.e. approximately 30 µmoles per rat, may be sufficient to increase CPH concentration in blood stream up to the comparable level. Since CPH appears to impair Ca flux in islet cells[15], the leakage of insulin caused by CPH might be due to the disorder of membrane integrity of beta cells or impairment of insulin storing process. The abnormal histologic findings of intracellular organelle in CPH-treated islet cells support this possibility.

Finally we were not able to detect an increase of insulin in plasma of CPH-treated rats. Since CPH appears to affect beta cells quite rapidly, sequential monitoring of plasma insulin levels in the early phase after CPH administration must be carried out in order to reach definite conclusions.

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Serum-induced stimulation of snRNA synthesis in mouse 3T3 fibroblasts

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Summary. Small nuclear RNAs (snRNAs) from quiescent and serum-stimulated 3T3 cultures, labeled with [^{3}H]uridine ([^{3}H]U), were electrophoresed in polyacrylamide-urea slab gels and revealed by staining with ethidium bromide and by fluorography. Judged by labeling with [^{3}H]U, synthesis of 7S and U1–U6 RNAs was very low or absent in quiescent cultures. The serum-induced transition of 3T3 cells from a resting to a growing state was accompanied by an early, apparently sequential stimulation of snRNA synthesis; stimulated synthesis of 7S, U1, U2, U3, U4 and U6 RNAs coincided in time with serum-induced stimulation of 45S pre-ribosomal RNA (pre-rRNA) and heterogeneous nuclear RNA (hnRNA) synthesis.
Key words. Mouse 3T3 fibroblasts; serum-induced mitotic stimulation; snRNA synthesis; polyacrylamide-urea slab gels; U1–U6 RNAs.

In eukaryotic cells a number of small (90–400 nucleotides, nt), metabolically rather stable[3–5] nuclear RNA species (snRNAs) have been characterized (reviewed in Busch et al.[6]) which account for about 0.5% of total RNA[4]. The uridylic acid-rich snRNAs U1, U2, U4, U5 and U6 are present in the nucleoplasm[7] in the form of snRNPs[8,9]; they are associated with hnRNPs[10–12] and can be immunoprecipitated with immune sera from patients with systemic lupus erythematosus[13,14]. At least U1 RNA seems to be involved in splicing of hnRNA[6,13]. U3 RNA which is found in the nucleoli[4,15], hydrogen-bonded to nucleolar 32S rRNA[5,15], is thought to play a role in the maturation of 45S pre-rRNA[5,6,15,16]. All U-RNAs are transcribed by RNA polymerase II and are capped[6].

Transition of mouse 3T3 fibroblasts from a resting to a growing state can be induced by the addition of fresh serum[11,17,18]. We showed previously that tRNA synthesis was 2–3-fold stimulated within 1 h after addition of 10% bovine serum, whereas stimulation of overall RNA (45S pre-rRNA, hnRNA and 5S RNA) and protein synthesis began around 4 h, followed 6–7 h later by serum-induced DNA replication[17]. In the present work we studied snRNA synthesis in quiescent and serum-stimulated 3T3 cultures, focusing attention on the U-RNAs.

Material and methods. Mouse 3T3 fibroblasts (Flow Laboratories) were rendered quiescent by incubating confluent cultures (4×10^4 cells/cm^3) for 3 days in reinforced Eagle's medium supplemented with 0.5% fetal calf serum (GIBCO). Mitotic

stimulation was induced by addition at time zero of fresh serum to a final concentration of 10%[17]. The cultures were labeled with [5-^{3}H]uridine (29 Ci/mmol; Amersham) at the concentrations and for the length of time indicated in the figure legends. Nuclear and cytoplasmic fractions were prepared by the use of 1% Nonidet-P40 in 0.25 M sucrose, 10 mM triethanolamine, 25 mM NaCl, 5 mM $MgCl_2$, pH 7.4 and the RNA was extracted with phenol at 65°C[19]. Aliquots of about 10 µg nuclear or cytoplasmic RNA (in 5 M urea and 0.03% xylene blue) were electrophoresed either in polyacrylamide (10% acrylamide)-urea (7 M) or in gradient polyacrylamide (2.2–15% acrylamide)-urea (8 M) slab gels[17,20]. Electrophoresis was carried out at 120 V/gel at 20°C until the xylene blue had migrated 13–15 cm. RNA was revealed by staining with ethidium bromide and photographed under UV light (366 nm) using Polaroid films type 55 P/N; the gels were then treated with EN^{3}Hance (NEN), dried and exposed to X-Omat (Kodak) films for the lengths of time indicated in the figure legends.

Results. Quiescent and serum-stimulated 3T3 cultures were labeled with [^{3}H]U from 0–18 h; nuclear and cytoplasmic RNA was extracted at 18 h, subjected to electrophoresis in polyacrylamide-urea slab gels and analyzed by staining with ethidium bromide and by fluorography. The staining patterns of nuclear and cytoplasmic RNA extracted from quiescent (fig. 1a, b) and from serum-stimulated cultures (not shown), respectively, were indistinguishable. *Nuclear RNA* (fig. 1a) contained bands corresponding to 7S, U3, U2, U1, U4, U5 and U6 RNAs, in addition to 5.8S rRNA (doublet[5,21]), 5S RNA (doublet[4,15,22]) and traces of tRNA; furthermore, a faint doublet of an unknown snRNA containing 135–138 nt was observed. *Cytoplasmic RNA* (fig. 1b) contained 7S RNA[23] and U1 RNA[5,12]), in addition to 5.8S rRNA (singlet), 5SR RNA (doublet) and tRNA. The number of nucleotides of the snRNAs in 3T3 cells, observed by staining with ethidium bromide and/or by fluorography was estimated as shown in figure 2[24,25], taking as standards 5.8S rRNA (158 nt), 5S RNA (121 nt) and tRNA (80 nt)[6]; the 3T3 snRNAs are refered to according to the terminology proposed by Busch[6,21]. The band defined here as U1 RNA, migrating slightly slower than 5.8S rRNA, might correspond to U1b RNA observed in mouse ascites cells[13,14]. We also compared 3T3 snRNAs with U3, U2, U1, U4, RNAs, 5.8S rRNA and 5S RNA isolated from HeLa cells (a gift of Dr M. Jacob, Strasbourg) by electrophoresis either in polyacrylamide-urea or in gradient polyacrylamide-urea slab gels. The results showed that in both types of gels U2, U1 and U4 RNAs from 3T3 and HeLa cells comigrated, whereas U3 RNA from 3T3 cells migrated slightly faster, confirming a previous report[26]. Fluorography of nuclear RNA from quiescent cultures (fig. 1c) revealed that U3, U5 and 7S RNAs remained unlabeled and that U2, U1, U4 and U6 RNAs were very weakly labeled (fig. 3g), if at all (fig. 1c); in cytoplasmic RNA (fig. 1e) from quiescent cultures no label could be detected in 7S and U1 RNAs while 5.8S rRNA, 5S RNA and tRNA were, as expected [17], weakly, but distinctly, labeled. In nuclear RNA from serum-stimulated cultures (fig. 1d and 3h) all snRNAs, 5.8S rRNA and 5S RNA were labeled whereas only traces of radioactive tRNA were present. Cytoplasmic RNA from serum-stimulated cultures contained labeled 7S and U1 RNA, 5.8S rRNA, 5S RNA and tRNA[17].

To determine the onset of serum-induced snRNA synthesis, 3T3 cultures were labeled with [^{3}H]U for different length of time after addition of serum and nuclear RNA was analyzed by electrophoresis in polyacrylamide-urea slab gels (fig. 3). Judged by fluorography, serum-induced synthesis of U2 RNA, 5.8S rRNA, 5S RNA and pre-tRNA was observed after labeling from 0 to 3 h (fig. 3b) and synthesis of U3, U1, U4, U6 and the 135–138 nt snRNAs after labeling from 0 to 6 h (fig. 3d). Synthesis of U5 RNA and of two additional snRNA species comprising 90 and 97 nt were only observed after labeling from 0 to 10 h (fig. 3f); the latter two snRNAs, detectable by

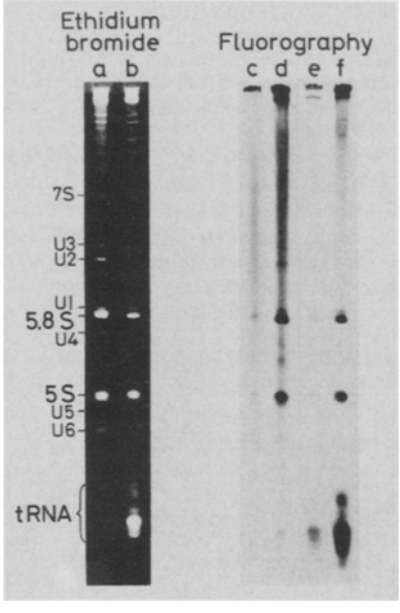

Figure 1. Analysis of nuclear and cytoplasmic RNA from quiescent and serum-stimulated 3T3 cultures by electrophoresis in a polyacrylamide-urea slab gel. Quiescent (a, b, c, e) and serum-stimulated (d, f) 3T3 cultures were labeled with 5 µCi/ml [5-^{3}H]U from 0 to 18 h after addition of 10% serum. RNA was extracted with phenol from nuclear (a, c, d) and cytoplasmic (b, e, f) fractions; aliquots of 10 µg were electrophoresed in a polyacrylamide (10% acrylamide)-urea (7 M) slab gel and RNA was revealed by staining with ethidium bromide and by fluorography (1-day exposure). Staining patterns of nuclear and cytoplasmic RNA from quiescent (fig. 1a, b) and from serum-stimulated 3T3 cultures (not shown) were indistinguishable. The bands above 7S RNA might correspond to the snPI RNAs described in Benecke et al.[27].

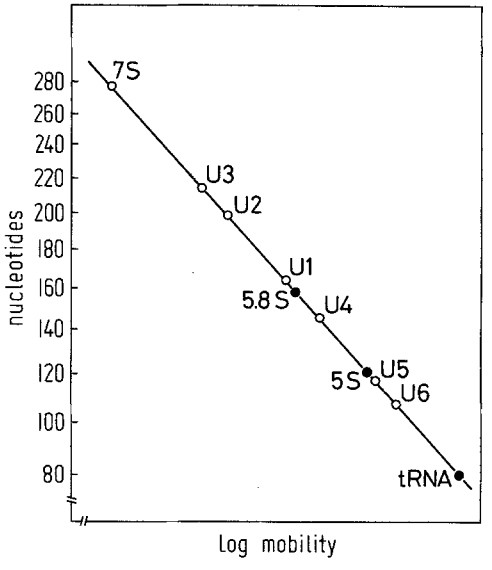

Figure 2. Estimation of the nucleotide number of 3T3 snRNAs. The measurements were based on the linear relation between the logarithm of the electrophoretic mobility and the square root of the number of nucleotides[24,25]. The standard curve (●) was obtained from 5.8S rRNA (158 nt), 5S RNA (121 nt) and tRNA (80 nt)[6]. The mobilities of 3T3 snRNAs (○) were measured on ethidium bromide-stained gels and/or on fluorographs. The estimates for 3T3 snRNAs were: 7S, 276 nt; U3, 214 nt; U2, 198 nt; U1, 163 nt; U4, 144 nt; U5, 113 nt and U6, 106 nt. Standard deviations for the U-RNAs, determined in three independant experiments, were less than 3%.

fluorography only, may correspond to La 4.5S and La 4.5SI RNAs, respectively[6].

In other experiments quiescent and serum-stimulated cultures were pulse-labeled with [3H]U from 4 to 5 h, i.e. shortly after onset of serum-induced stimulation of overall RNA and protein synthesis[17]. Total RNA was extracted and analyzed by electrophoresis in polyacrylamide-urea slab gels. The staining patterns of RNA from quiescent and serum-stimulated cultures (not shown) were indistinguishable and were closely similar to those obtained with RNA from isolated nuclei (fig. 1a, b), with the exception of the presence of cytoplasmic tRNA. Determined by fluorography, 7S and all U-RNAs from quiescent cultures (not shown) remained virtually unlabeled. In RNA from serum-stimulated cultures 7S, U3, U2, U4, U6 RNAs, 5.8S rRNA, 5S RNA and pre-tRNA were distinctly labeled whereas U1 RNA remained unlabeled, a finding in accordance with the results obtained by cumulative labeling (fig. 3). The presence of relatively large amounts of radioactive pre-tRNA, due to the pulse-labeling (60 min) of the cultures[17], precluded the detection of the 90 and 97 nt snRNAs.

Addition of 10% bovine serum to quiescent 3T3 cells stimulates within minutes the uptake of exogenous radioactive uridine and leads to increased labeling of the cellular RNA[17]. This cannot account, however, for the increase in labeling of the snRNAs reported here since the onset of stimulated synthesis was asynchronous and took place at apparently different rates (compare e.g. U3 and U4 RNA in fig. 3g and h).

Discussion. Phenol-extracted RNA from quiescent and serum-stimulated 3T3 cultures was analyzed by electrophoresis in polyacrylamide-urea slab gels. Determined by staining with ethidium bromide, nuclear RNA preparations contained 7S RNA, U1-U6 RNAs, 5.8S rRNA, 5S RNA, traces of tRNA and a 135–138 nt snRNA doublet; cytoplasmic RNA preparations contained 7S RNA, U1 RNA, 5.8S rRNA, 5S RNA and tRNA. The electrophoretic patterns of snRNAs from quiescent and serum-stimulated 3T3 cultures were indistinguishable and were very similar to those observed in other mammalian cells[7, 21, 27]. These results also showed that quiescent 3T3 cells contain a pool of snRNAs. To estimate apparent rates of snRNA synthesis, 3T3 cultures were labeled with [3H]U and radioactive RNA was revealed by fluorography of the gels. Estimated by cumulative labeling with [3H]U for 18 h, synthesis of the snRNAs was very low or absent in quiescent cultures, whereas in serum-stimulated cultures all snRNAs were distinctly labeled; the latter result is in agreement with the observation[26] that incubation of growing 3T3 cultures with [32P] or [3H]U for 24-h periods led to the labeling of seven radioactive snRNAs.

The results reported here show that serum-induced transition of 3T3 cells from a quiescent to a growing state comprises an early, apparently sequential stimulation of snRNA synthesis; the observation that stimulated synthesis of U1, U2, U3, U4 and U6 RNA coincides in time with the onset of serum-induced stimulation of 45S pre-rRNA and hnRNA synthesis is of interest since snRNAs are thought to play a role in splicing of hnRNA[6, 13] and in the maturation of 45S pre-rRNA[5, 6, 15, 16].

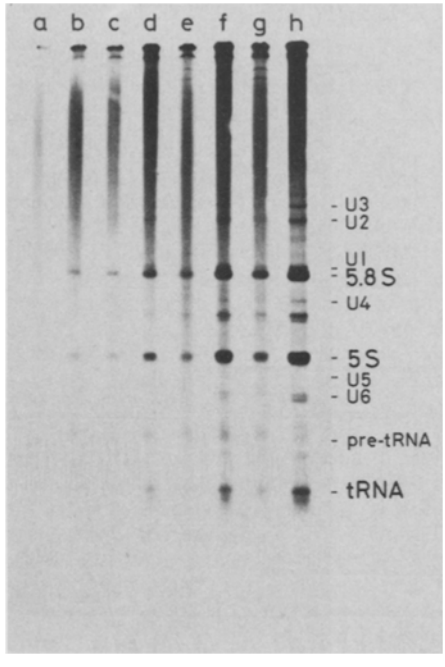

Figure 3. Time-course of serum-induced stimulation of 3T3 snRNA synthesis. Quiescent (a, c, e, g) and serum-stimulated (b, d, f, h) cultures were labeled with 5 μCi/ml [5-3H]U from 0 to 3 h (a, b), 0 to 6 h (c, d), 0 to 10 h (e, f) and 0 to 18 h (g, h) after addition of 10% serum. Aliquots of 10 μg nuclear RNA were electrophoresed in a polyacrylamide (10% acrylamide)-urea (7 M) slab gel. RNA was revealed by staining with ethidium bromide and by fluorography (2-day exposure). The staining patterns were indistinguishable from those shown in figure 1a and were therefore omitted.

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