sults in changes in central nervous system activity, these treatment related regional patterns may represent one aspect of this adaptation process, wherein greater increases in blood flow compensate for commensurate increases in regional neuronal activity.

The distribution of a population of opioid receptors in the brain has been delineated in a number of species, including the rat, using dihydromorphine[17]. One region with a high density of these opioid receptors in the rat is the midbrain. Another region with a relatively dense opioid receptor population is the cortex. Interestingly, we found that these regions underwent the greatest increase in %CO at 15 min post-200 mg/kg, i.v., morphine. In contrast, those regions which underwent the greatest increase in %CO 48 h after continuous morphine administration, the pons and cerebellum, are regions which tend to have a more sparse opioid receptor population in the rat. Recently, a number of different opioid receptors have been identified in brain. It would be of interest to compare the density of these receptors in various brain regions with changes in blood flow using appropriate agonists to those receptors.

Finally, the present data suggests that both acute and chronic morphine administration alters rCBF, in contrast to earlier statements suggesting no change following morphine treatment[8]. Therefore, further studies are needed to characterize the mechanisms by which opioids affect blood flow to brain and regional cerebral blood flow.

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14 Mean left ventricular blood pressures:

	Acute	Chronic
Control	49.6 ± 1.47	57.4 ± 1.93
Post-morphine	48.8 ± 1.62	52.9 ± 2.12

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The effect of cyproheptadine on insulin biosynthesis

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Summary. The effect of a potent antiserotonin-antihistaminic compound, cyproheptadine (CPH) on insulin biosynthesis was studied in pancreatic islets isolated from CPH-treated rats. Though insulin content of islets was markedly reduced in CPH-treated rats, the incorporation of radiolabeled leucine into proinsulin and insulin fractions was not affected with respect to the rate and amount. It is concluded that CPH may deplete insulin content of the islets through causing the leakage of insulin.
Key words. Pancreatic islets, rat; insulin biosynthesis; cyproheptadine.

Cyproheptadine (CPH), a potent antiserotonin-antihistaminic compound, has been reported to cause glucose intolerance in rats when given orally in large dose[3,4]. The morphological investigation of these rats revealed selective abnormalities of pancreatic beta cells such as degranulation and vacuolization of rough endoplasmic reticulum. In addition CPH caused a remarkable reduction of insulin content of pancreatic islets in a few days of the treatment[5]. These results suggest the specific effect of this compound on pancreatic beta cells. Though there have been reports indicating the inhibitory action of CPH on insulin secretion[6,7], its effect on biosynthetic process of insulin has not been clarified yet. The present study was conducted to investigate the effect of CPH on insulin biosynthesis using pancreatic islets isolated from CPH-treated rats.

Materials and methods. Animals. Male albino rats (Wistar strain) weighing 150–200 g were fed with regular laboratory chow and water. Cyproheptadine-hydrochloride (Sharp and Dohme, Munchen, FRG), in aqueous solution, was given by gastric intubation at a dose of 45 mg/kg b.wt for 1, 2, 10 or 20 consecutive days.

Insulin biosynthesis studies. Groups of 30 islets isolated from cyproheptadine (CPH)-treated rats by collagenase digestion technique[8] were incubated with 1 ml of Krebs-Ringer-bicarbonate (KRB) buffer containing 0.5% bovine serum albumin, 11 mM glucose, 10 µg of amino acids mixture without leucine and 5 µCi of L-(U-^{14}C)-leucine or 100 µCi of L-(4,5-^{3}H)-leucine (The Radiochemical Centre, Amersham, U.K) as tracer. The incubation was performed for 30, 120 or 240 min at 37 °C in the atmosphere of 95% O_2, 5% CO_2. After incubation the islets were washed with ice-cold KRB buffer and extracted with acid-ethanol[9]. The extracts were then dried in a stream of air, dissolved in 3 M acetic acid and chromatographed on a 1 × 50 cm column of Biogel P-30 (Bio-rad Labs, Richmond, Calif.) previously equilibrated with 3 M acetic acid[10].

Analytical method. Immunoreactive insulin (IRI) was measured in acid-ethanol extracts of the islets and in each fraction of column eluates according to the method of Herbert et al. as previously reported[11,12]. The measurement of radioactive leucine incorporation was performed in eluate samples mixed with Aquasol (New England Nuclear, U.K.) using a liquid scintillation spectrometer (LS-330, Beckman Instruments, Fullerton, Calif.).

Results. 1. Insulin content of isolated islets (fig. 1). IRI level measured in acid-ethanol extracts of islets were significantly decreased in all groups of CPH-treated rats. The change appears remarkably rapid, being already obvious 12 h after a single dose (45 mg/kg) of CPH. The IRI levels showed a rebound increase (10 and 20 days-treated rats) after reaching the nadir on the second day though the levels remained significantly lower than controls. These results were in accord with those obtained with whole pancreas of CPH-treated rats as previously reported[11].

2. Insulin biosynthesis. Acid-ethanol extracts of control islets incubated with ^{14}C-leucine for 30, 120 and 240 min were chromatographed on Biogel P-30 column and the elution pattern of radioactivity and IRI are shown in figure 2. The peak of IRI was identified as insulin by eluting ^{125}I-insulin on the same column and the profile was not different after different incubation times. The radioactive profiles shown were similar to those reported by others[13]. In the early phase of incubation the radioactivity was incorporated mainly into proteins larger than insulin and gradually moved to smaller one coincided with the insulin fraction.

The islets extracts obtained from CPH-treated rats were also chromatographed on the same column. The elution profiles of radioactivity and IRI in both 2 and 20 days-treated rats were not different from those of control rats in terms of rate and pattern, except for the lower IRI activity in CPH-treated rats (data not shown). In an attempt to detect minor abnormalities of elution profiles the acid-ethanol extracts of ^{3}H-labeled control islets and ^{14}C-labeled CPH-treated islets (0, 2 and 20 days)

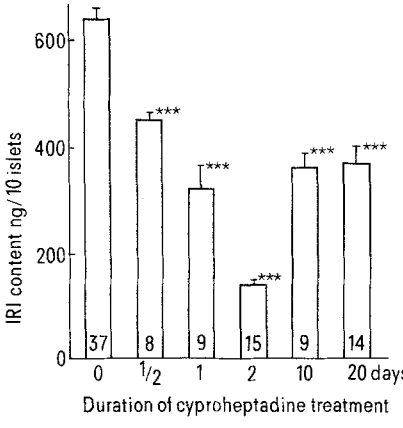

Figure 1. Insulin (IRI) content of isolated islets obtained from CPH-treated rats. Acid-ethanol extracts of the islets were provided for IRI assay. Values are mean ± SEM. Figures inside the bar are number of rats in each group. *** $p < 0.001$ vs control (0 day) values by t-test.

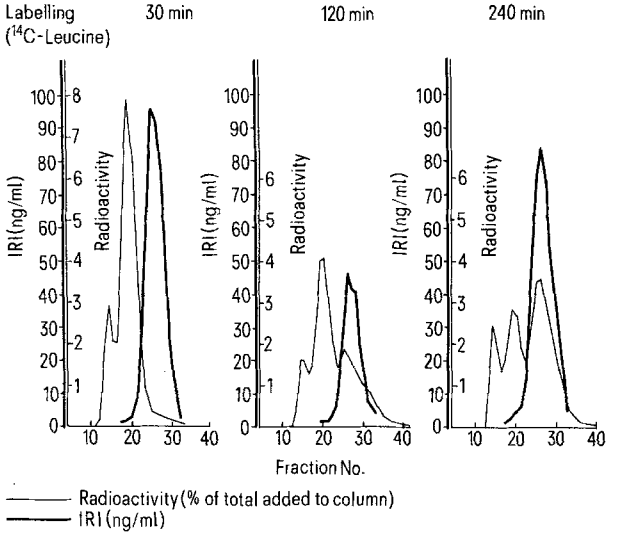

Figure 2. Elution patterns of radioactivity and IRI resulting from the chromatography of acid-ethanol extracts of control islets incubated with ^{14}C-leucine for 30, 120 and 240 min. The chromatography was performed on Biogel P-30 column (1 × 50 cm). The radioactivity was expressed as percent value of totals applied to the column.

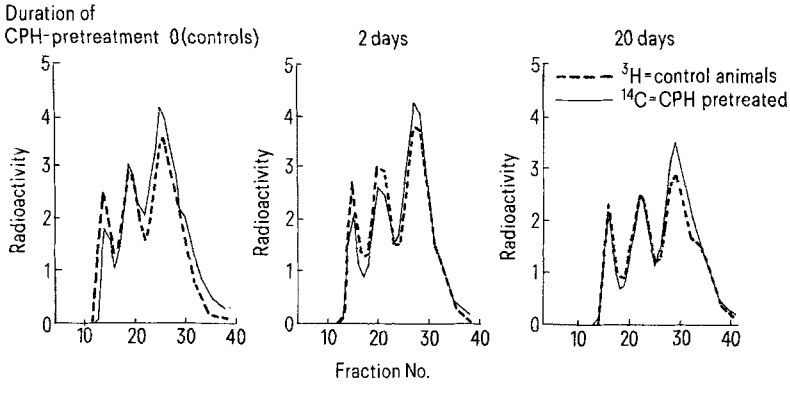

Figure 3. Radioactivity profiles of acid-ethanol extracts of islets on Biogel P-30 column (1 × 50 cm). Control islets were incubated with ^{3}H-leucine and CPH-treated islets incubated with ^{14}C-leucine for 240 min. The extracts of both islets were mixed together prior to applying the column.

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