olar fibrillar centres, would be an aggregation of cistrons of the nucleolar chromatin loop not engaged in active transcription (Figure 4). The clear areas surrounding them would correspond to ribosomal cistrons in active transcription; their low electronic density, their loose structure and their small size (10 Å) would be due to the fact that nascent RNA molecules would be short with few associated proteins. The fibrillar component zone contiguous with the clear areas would correspond to ribosomal cistrons with long RNP molecules; hence also a transcription zone.

In the fibrillar centre zones most distant from the clear areas, the RNP molecules seem to be further liberated from the ribosomal cistrons. The maturation process of the ribosomal precursors would seem to start at this stage; the molecules undergo a morphological condensation process, becoming shorter and shorter, globular subunits (90 Å) are seen in them and they subsequently undergo a coiling process, giving rise to the 150–200 Å granules.

We may therefore consider each nucleolar fibrillar centre as an active zone in the nucleolar chromatin loop (Figure 4), where first transcription and then maturation of the ribosomal precursors take place. All the active fibrillar centres together form a single organelle: the nucleolus, with all the fibrillar centres immersed in the granular component, which is their common final product.

Beta-Adrenergic Receptors in Rat Myocardium: Direct Detection by a New Fluorescent Beta-Blocker

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Summary. A new fluorescent β-blocker, 9-amino-acridin propranolol (9-AAP), was administered i.v. to rats. Multiple fluorescent 9-AAP binding sites were observed on cardiac muscle cells in frozen sections. Intensity and density of cardiac 9-AAP fluorescence were markedly reduced following pretreatment with (±)- and (−)-propranolol but not with (+)-propranolol. Our findings suggest that 9-AAP may label β-adrenergic receptor sites in rat myocardium.

Since the initial classification of adrenergic receptors[2], considerable evidence has become available to indicate the presence of β-adrenergic receptors within the mammalian heart. The identification and characterization of cardiac β-adrenergic receptors have relied mainly on pharmacological[3], electrophysiological[4], and biochemical[5] approaches. In addition, radioactively labeled β-adrenergic agonists[6] and antagonists[7] were used in vitro to identify β-receptor sites in cardiac preparations. Although various methods are available, a more direct approach for the detection of β-receptors within the myocardium is warranted. Preferably such a method would permit an in vivo study, whereby possible alterations in the properties of the β-receptors induced by in vitro preparations of cardiac tissues may be avoided or minimized.

Recently, a potent fluorescent β-adrenergic antagonist, 9-amino-acridin-propranolol (9-AAP) has been synthetized[8]. This compound is a fluorescent analogue of propranolol and its chemical structure is (*N*-[-2-hydroxy-3-naphthoxy propyl]-*N'*-[9-amino-acridin]isopropyl diamine) (Figure 1). The spectroscopic molar extinction coefficient of 9-AAP, ε_{260} in water, is 1.07×10^5. The inhibitory effect of 9-AAP on β-adrenergic receptors was calculated from the concentration of this compound which was required to inhibit 50% of the (−)-epinephrine stimulated activity in a β-receptor-dependent adenylate cyclase system[9,10]. The dissociation constant of 9-AAP to the β-adrenergic receptor was found to be $(3 \pm 1) \times 10^{-8}$ *M*[8,11].

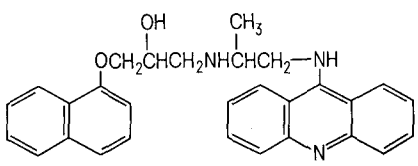

Fig. 1. The structure of 9-amino-acridin propranolol.

We have recently used 9-AAP to localize β-adrenergic receptors in rat cerebellum[11]. The present in vivo study was designed in an attempt directly to detect β-adrenergic receptors in rat myocardium by the utilization of 9-AAP as a fluorescent probe.

Material and methods. 9-AAP in saline (2.5 mg/kg) was administered by slow injection into the tail veins of albino rats (200–220 g). Control animals were pretreated with one of the following compounds: (±)-propranolol, (−)-propranolol, or (+)-propranolol (5 mg/kg in saline) by slow i.v. injection. 30 min later, 9-AAP (2.5 mg/kg) was administered to each of the control animals. All animals were killed by decapitation under light ether anaesthesia 30 min after injection of 9-AAP. The heart of each animal was quickly removed, immersed in 'Tissue OCT Compound' (Ames, USA) and frozen in liquid nitrogen. Later, 6–8 μm cardiac sections were cut in a cryostat

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at −20 °C. The frozen sections were mounted on glass slides and air-dried. Sodium phosphate buffer, 0.08 *M*, pH 7.4, was applied and coverslips were placed in position. The sections were visualized under phase-contrast and transmitted ultraviolet illumination on a Zeiss Universal Fluorescent Microscope with HBO 200 W super pressure mercury lamp, exciter filter U.G.1 and barrier filter No. 41.

Results. Multiple yellow, intensely fluorescent dots were observed in cardiac sections from animals which were treated by 9-AAP alone (Figure 2). Similar pattern and intensity of fluorescence were noted in atrial, ventricular, subendocardial and septal myocardial sections. Fluorescent dots were practically absent in the endocardium and epicardium. Dotted fluorescence was often noted to be arranged as threads or chains consisting of several fluorescent beads and spots which were localized mainly on membranes of cardiac muscle cells. This distribution pattern of 9-AAP fluorescence was observed in longitudinal as well as in cross sections through cardiac muscle cells (Figure 3).

In addition to the well defined 9-AAP yellow fluorescent dots, this compound also coloured the myocardial membranes with a diffuse non-specific greenish tint. Dull opaque non-specific greenish fluorescence was occasionally observed in erythrocytes, dispersed among cardiac muscle cells.

Dotted fluorescence was reduced, and sometimes even totally absent in cardiac sections from control animals pretreated with either (±)-propranolol or with (−)-propranolol. The density and intensity of 9-AAP fluorescence in cardiac sections from control animals pretreated with (+)-propranolol were higher than in sections obtained from animals pretreated with (±)- and (−)-propranolol. The degree of fluorescence, however, was moderately lower in sections from (+)-propranolol pretreated animals as compared with cardiac preparations from rats treated by 9-AAP alone.

Discussion. Our findings suggest that 9-AAP binding sites may represent β-adrenergic receptor sites in rat myocardium. The marked affinity of propranolol to β-adrenergic receptors is well established. Its fluorescent analogue, 9-AAP, also exhibits high affinity to β-receptors when studied in vitro with a β-receptor-dependent adenylate cyclase system[8,11]. Furthermore, binding of propranolol to cardiac β-receptors is stereospecific[12]. In the present study, both the intensity and density of fluorescence were lower within the myocardium of control animals pretreated with (±)- and (−)-propranolol, as compared to those pretreated with the (+)-racemic isomer. Though evaluation of fluorescence was qualitative, stereospecific blocking of 9-AAP binding sites may be inferred from these findings. Fluorescence in animals pretreated with (+)-propranolol was reduced as compared with that observed in sections from rats treated by 9-AAP alone. This phenomenon is possibly due to low specific activity of the (+)-racemic isomer or to its contamination with small amounts of (−)-propranolol[13].

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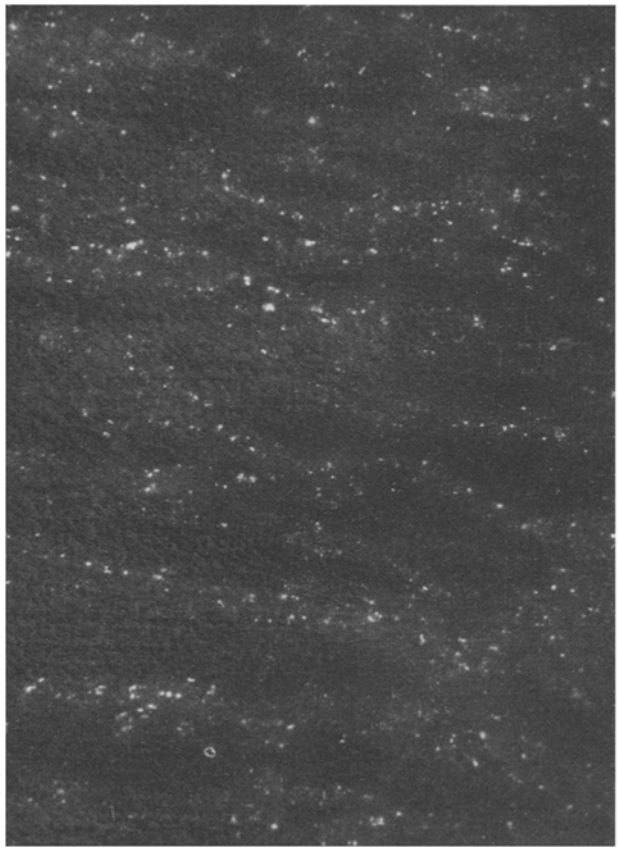

Fig. 2. Fluorescence photomicrograph of longitudinal section through rat ventricular myocardium. Chains of fluorescent dots are observed on cardiac muscle cells. Frozen section. ×60.

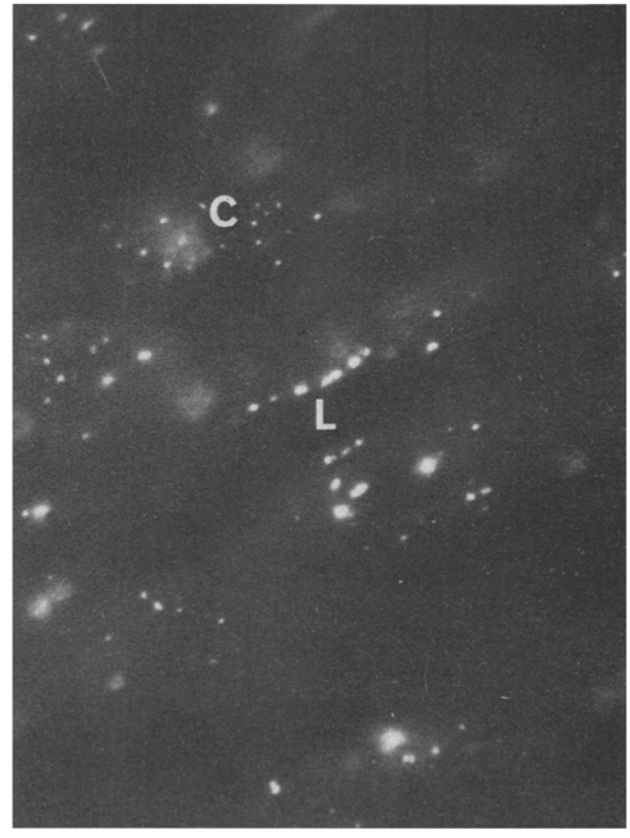

Fig. 3. High magnification fluorescence photomicrograph showing fluorescent beads on membranes of longitudinal (L) and cross (C) sections through cardiac muscle cells. Frozen section. ×200.

Apart from specific binding to β-adrenergic receptors, propranolol also exhibits non-specific, low affinity binding to cardiac membranes[14]. The diffuse greenish colouring of the cardiac sections may represent non-specific 9-AAP binding to myocardial membranes.

Recent indirect evidence suggests that the β-adrenergic receptors are localized in the cardiac cell membrane[15–17]. This view may be supported by the present findings. Furthermore, the observed distribution pattern of dotted fluorescence may represent discrete areas with high concentration of 9-AAP binding sites in the myocardial membrane. By inference, it may be suggested that the β-adrenergic receptor sites are not evenly distributed in rat cardiac cell membrane.

More evidence must be obtained to establish a definite analogy between 9-AAP binding sites and β-adrenergic receptor sites. Utilization of 9-AAP may provide a useful tool for the direct detection of β-adrenergic receptors in the heart and also in extra-cardiac tissues.

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Preparation of Isolated Cells from Rat Heart

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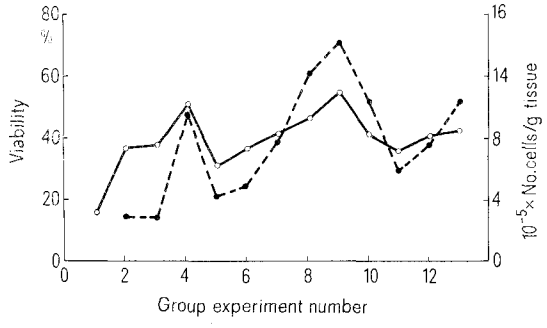
Summary. Suspensions of isolated cells from rat heart were prepared and the data for viability and yield are given. Glucose uptake by the cells was mediated by a carrier system.

Heart tissue has been successfully dispersed into preparations of viable, isolated cells[2–5], but only the last authors[5] have discussed systematically the methodology. However, some important quantitative aspects were lacking in their paper (e.g. statistics of viabilities and their relation to yields); and furthermore, the very high percentage viabilities they obtained are probably unrepresentative of the method, and could lead to the rejection of otherwise acceptable preparations.

Effect of temperature of incubation with collagenase on the preparation of suspensions of isolated cells from rat heart

Harvest 3		Harvest 4	
Viability (%)	Cell yield[a]	Viability (%)	Cell yield[a]
30 °C 55.2±3.8(5)[b]	1000±264(5)	62.8±4.6(5)[c]	1397±416(5)
37 °C 40.2±1.8(13)[b]	939±147(13)	39.3±2.1(13)[c]	1026± 95(13)

Cells harvested at 20 min intervals.
[a] 10^{-3} × yield of live cells per g heart tissue.
[b] Significance of difference $p < 0.005 > 0.001$.
[c] Significance of difference $p < 0.001$.



Percentage viability (○—○) and numbers of cells/g tissue (●--●) are given for all comparable suspensions of heart cells prepared in Harvest 3 in these experiments. The data are means for successive groups each of 3 experiments. Harvest 3 is the suspension of cells dispersed during the 3rd 20 min period of incubation with collagenase.

Methods. Male albino rats (100–120 g) were stunned and decapitated. The cardiac ventricles were diced (side 3 mm). Tissue (< 2 g) was incubated at 37 °C in a 50 ml conical flask containing 3.5 ml of a phosphate-buffered medium (pH 7.4) with collagenase (1 mg/ml). The flasks were shaken at 100 strokes/min. The composition of the medium (g/l) was: 8.8 g NaCl, 0.4 g KCl, 0.21 g Na_2HPO_4, 0.06 g NaH_2PO_4 and 0.9 g glucose. Cells were decanted from the tissue pieces at 20 min intervals (harvests 1–4) and the tissue resuspended in fresh buffer. Harvests 1 and 2 were mostly erythrocytes and fragmented cells and were discarded. Cells from harvests 3 and 4 were collected by centrifugation (60 × *g*, 2.5 min), washed 3 times and counted (cytometer depth 0.2 mm). Viability was determined by staining with trypan blue (equal volume 0.25% dye). Purification of cells by centrifugation through Ficoll (30 g/l)[5] was omitted because it a) rarely improved percentage viability; b) always reduced yields; and c) did not change the microscopic appearance.

For metabolic experiments the medium contained lower glucose (0.5 m*M*), [U-^{14}C]glucose (0.625 μCi/ml), bovine serum albumin (1 g/l) and $CaCl_2$ (2.5 m*M*). Cell viability was not affected by incubation for 60 min. The optimum number of cells per incubation sample (1 ml) was 3–4 × 10^5. With cell numbers of 5 × 10^5 filtration was difficult. Incorporation of ^{14}C into cells was determined by scintillation counting of washed cells on a Millipore filter (1.2 μm, 0.25 mm) in a scintillation mixture (Triton X-100: toluene: POPOP). Comparisons are between cell

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