through a 200 gauge stainless steel screw press. The pulp devoid of most of the fibrous content of the tumour, was suspended (1 g in 5 ml) in a hypotonic solution of 1/5 TKM (0.05 *M* Trizma pH 8.2, 0.025 *M* KCl and 0.005 *M* $MgCl_2$) and left for 15 min. The suspension was spun at 1000 g for 10 min and the supernatant containing lysed red blood cell products discarded. The residue was resuspended in 1/5 TKM and passed several times through a 23 gauge hypodermic syringe needle until cell disruption was complete and nuclei released as judged by phase contrast microscopy. This nuclear suspension was either spun at 1000 *g* for 10 min or at 100,000 *g* for 40 min through a layer of 2.3 *M* sucrose. These last two procedures supplied pellets of nuclei contaminated with other cellular products (low *g*) or pellets free of contamination with soluble cytoplasmic enzyme (high *g* through sucrose). The nuclei were finally resuspended in 1/5 TKM, warmed to 37 °C and mixed with an equal volume of nucleoside triphosphate solution at 37 °C. The number of nuclei at this stage varied between 1–5 $\times$ 10^7 per ml.

Nucleoside triphosphate mixture. The ingredients of the DNA precursor triphosphate mixture were as described by LYNCH et al.[13]. The tritiated pyrimidine or purine nucleoside triphosphates were used at 4 µCi per ml, specific activity 20–22 Ci/mmol.

DNA synthesis reaction. A magnetic stirrer was used to ensure even distribution of nuclei in the reaction mixtures. 1 ml samples were taken at appropriate time intervals and added to 2 ml ice-cold 10% trichloro acetic acid. After 18 h the TCA precipitated residues were washed by centrifugation in TCA and finally filtered onto glass fibre filters. The activity retained on the filter, representing newly synthesized DNA, was counted in standard xylene–Triton X 100, POP-POPOP, scintillant fluid. Throughout these experiments 1000 cpm is equivalent to 45.3 picomoles of ^3H pyrimidine in acid insoluble material; or equivalent to 10^{14} nucleotides in newly synthesized DNA.

Estimation of DNA. Duplicate samples taken at the same time as those used to follow the formation of acid insoluble reaction products were used to determine total DNA. The technique described by BURTON[14] was used.

Results and discussion. Figure 1 illustrates a typical reaction curve. Synthesis was characterized by an initial formation of acid precipitable newly formed DNA which reached a peak after 50–60 min. followed by a steady decline in precipitable radioactivity. This pattern of increase and eventual loss in activity was consistently repeated in many reactions of nuclear synthesis which we followed with nuclei from the RIB_{5c} tumour and other tumour lines 66DT and SSB_1 (ref. [12]).

Figure 2 compares the pattern of synthesis by isolated nuclei where they were finally purified either at low speeds in hypotonic solution or at high speed through 2.3 *M* sucrose. The sucrose purification technique reduced the total amount of new DNA synthesized and the rate at which degradation subsequently occurred.

We were surprised to find that DNA synthesis by intact nuclei from the rat tumour RIB_{5c} exhibited a peak of activity followed by considerable degradation of the newly formed DNA. However, most previous workers have reported only on the initial incorporation of triphosphate precursors and it is not clear from the published work whether the early formed product is stable in the continued presence of the reaction mixture. The degradation was much greater than the breakdown of the existing nuclear DNA as assessed by BURTON's technique, indicating that degradation processes are specific for the newly formed DNA. In similar experiments with mouse lymphoma cells and chinese hamster fibroblasts we also observed extensive degradation after early synthesis.

One interpretation of these results is that nuclei isolated by the methods generally used, contain firmly attached enzymes responsible for both synthesis and degradation of DNA. Under the reaction conditions used by ourselves and many other workers this degradation must be considered in assessing the activity of the nuclei.

From this initial work we feel that the synthetic behaviour of nuclei isolated from solid neoplasms can be used to investigate further the effects of drugs and other cytotoxic treatments on the nuclei individually or perhaps on the tumour structure as a whole.

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Ultrastructural Aspects of Nucleolar Fibrillar Centres in Meristematic Cells of *Allium cepa*

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Summary. This paper deals with the fine structure of the fibrillar centres of the nucleolus in *Allium cepa* cells in ultrathin, sections of in vivo fixed roots. The ultrastructural observations have allowed us to consider each nucleolar fibrillar centre as an active zone in the nucleolar chromatin loop, and to propose a possible model for the organization of the different components of the nucleolus within it.

The structure of the nucleolus in meristematic cells of *Allium cepa* has been described by electron microscopy on numerous occasions[2,3]. Three components are clearly distinguished: fibrillar, granular and chromatin; each with a very precise location within the nucleolus. The fibrillar component is densely packed and forms zones of more or less irregular appearance, which are interconnected and immersed in the granular component. We have called them fibrillar centres. This study deals with the fine ultrastructure of these nucleolar fibrillar centres in ultrathin sections of material fixed in situ.

Material and methods. The material used consisted of root-tip meristematic cells from *Allium cepa* L. bulbs grown in tap water under constant conditions of tem-

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perature and aerations (15 ± 0.5 °C) and bubbling 10–20 cm^3 air min^{-1}. The roots were fixed and stained as follows: Conventional fixing: fixation in 3% glutaraldehyde in a 0.025 *M* cacodylate buffer at pH 7 for 1 or 2 h, at room temperature, and postfixation in 1% osmic acid for 1 h in the same buffer. EDTA technique according to BERNHARD[4]. Fixation in 2.5% glutaraldehyde in Sorensen's phosphate buffer pH 7.3 for 1 h at room temperature. The roots were dehydrated with graded concentrations of ethanol, passed through propylene

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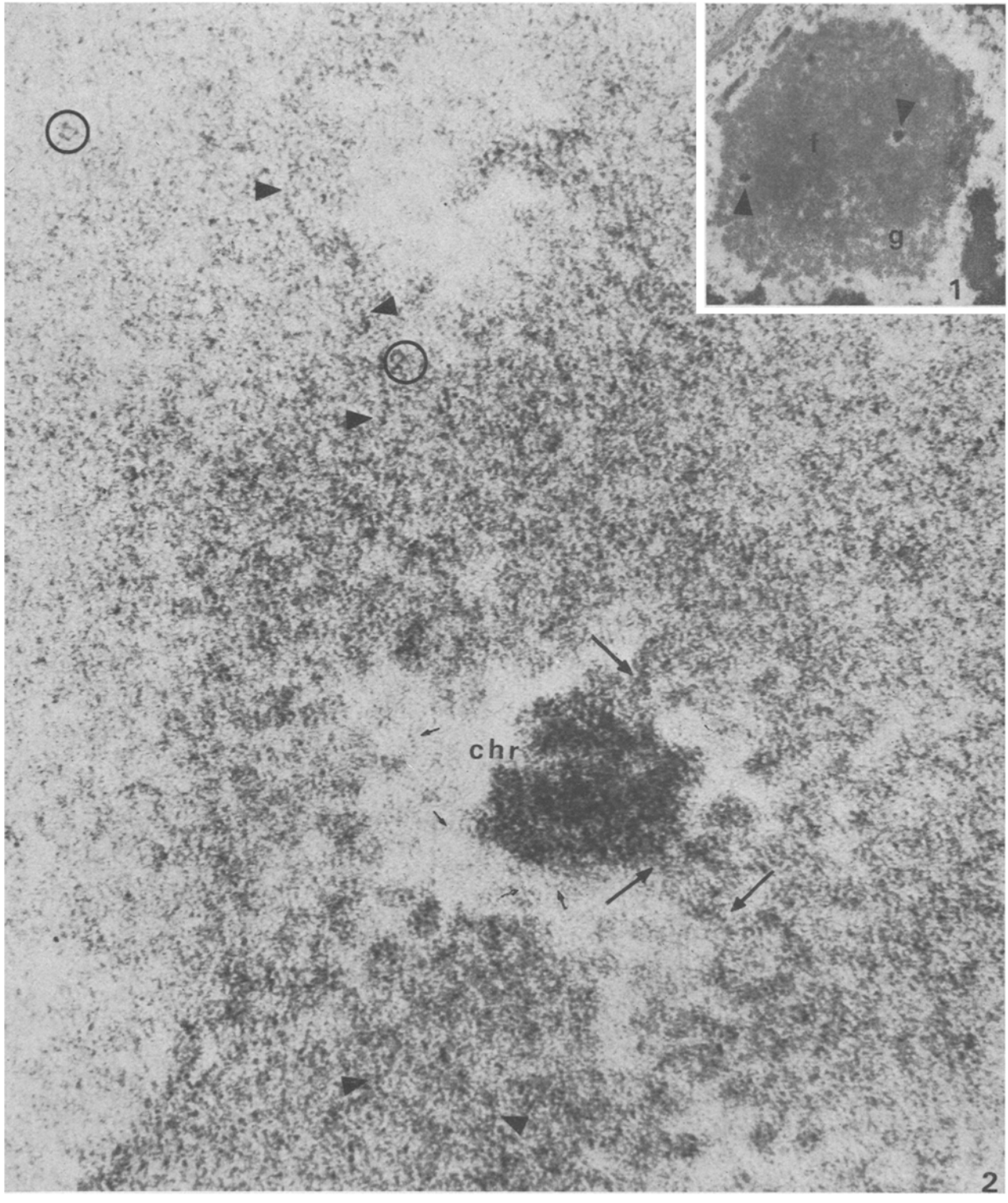

Fig. 1. Interphasic nucleolus: fibrillar part (f), granular part (g), intranucleolar chromatin (arrows). × 22,344.

Fig. 2. Fibrillar centre of the nucleolus in Figure 1. Pars chromosoma (chr) with its fibres in connection with the clear areas of the fibrillar component. Nucleolar RNP in several steps of processing (arrows, ○). Chromatin fibres with associated RNP (long arrows). × 307,800.

oxide and embedded in Epon 812. The sections from conventional fixations were stained with uranyl acetate in Michaelis and lead citrate, and sections for EDTA technique were stained as follows: 1. with 5% uranyl acetate in aqueous solution 45 sec; 2. EDTA in distilled water 35 min; 3. lead citrate, 45 sec.

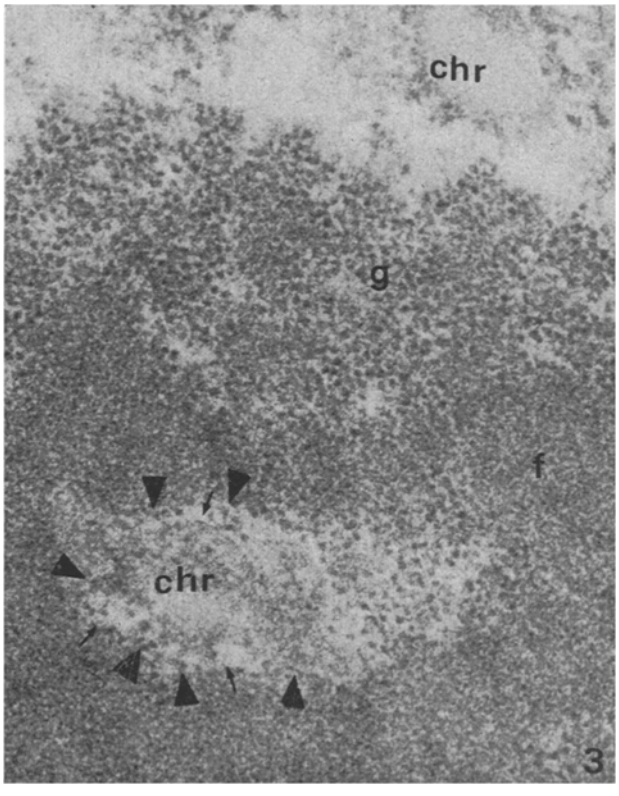

Fig. 3. EDTA. Bleached chromatin (chr), in the nucleus and in the clear areas of the fibrillar component (f) of the nucleolus. Granular component (g). Fibrils of RNP (arrows) contrasted in the clear areas of the fibrillar centres, the 10–15 Å fibrils in these zone appear unstained (little arrows). × 123,120.

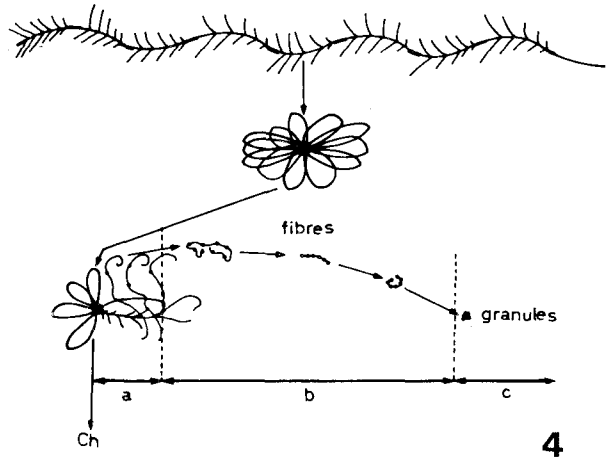

Fig. 4. Ultrastructural organization of the fibrillar centres of the nucleolus in *Allium cepa* meristematic cells. Ch, Intranucleolar condensed chromatin; a, lighter fibrillar zone: transcription and maturation of nucleolar RNP; b, fibrillar zone: maturation; c, granular zone.

Observations. Three main zones may be distinguished in nucleolar fibrillar centres from the centre outwards: 1. Dense chromatin zone, consisting of bundles 30–90 μm in diameter (Figure 1), which are in turn made up of thinner coiled fibres (100 Å). At great magnification these fibres are seen to be formed of tightly coiled 30 Å fibrills (Figure 2).

2. A zone, which, lighter to the electrons, surrounds the dense chromatin and is occupied by very low electronic density 10–15 Å fibrills, which are neither packed nor coiled (as are chromatin fibres or the fibrills in the nucleolar fibrillar component), but perfectly individualized. They run a very short distance across the section and both their ends are open, indicating that they must run across a number of planes so that we only detect those segments lying on the plane of the section. These fibres are continuous with the chromatin fibre bundles and with the 70–90 Å fibrills in the fibrillar component of the nucleolus. Granules, 15–20 Å in diameter offering greater contrast, are often seen aligned on the 10–15 Å fibrills. With EDTA these fibrills are bleached as in nucleolar and extranucleolar chromatin (Figure 3); this clear zone would then appear to be decondensed chromatin.

3. Fibrillar component zone. A different structure characterizes the part continuous with the 10–15 Å chromatin fibres, the central part, and the zone in contact with the granular component. In the first zone packing is much looser, and fibrills stretch long and sinuous across the section while lateral prolongations perpendicular to the axis of the fibre are seen (Figure 2). In the middle portion, the fibrills are more contracted, their length across the section is reduced and in them are seen globular subunits which give them a rosary-like appearance. In the final part, contrast is much greater and the fibres are often coiled up on themselves.

At great magnification, no clear separation between the fibrillar and granular components seems to exist; the fibrills become more and more tightly coiled, forming discrete packages: the granules with a diameter of 150 to 200 Å, while, between them, sections corresponding to the fibres are seen. These discrete packages have loose structure, in which the fibres forming them may be traced. At the periphery of the nucleolus, they become tighter and it is practically impossible to distinguish the fibres inside them.

Discussion. Our observations on the fibrillar centres of the nucleolus, at great magnification, strongly suggest that they constitute the active centres of transcription and processing of nascent rRNA on the most elementary level that we are able to study with the techniques used. At the same time, the granular component would correspond to fairly well structured ribosomal precursors.

The structural data obtained in this study are in essentially in agreement with those of other authors who have used spreading techniques[5–8] or isolation followed by biochemical and structural studies of the nucleolar components[9–11]. Thus the bundle of chromatin fibres, making up the so-called 'pars chromosoma' of the nucle-

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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].

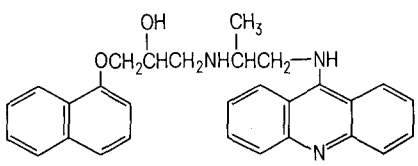

Fig. 1. The structure of 9-amino-acridin propranolol.

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

[1] D. A. is supported by a fellowship from the Lady Davis Fellowship Trust and by a Research Grant from the Bat-Sheva de Rothschild Fund for The Advancement of Science and Technology. The technical assistance of A. Itzhak is gratefully acknowledged.

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