It is noteworthy that in recent years considerable attention has been paid to glyoxalases since they are believed to participate in the regulation of cell division (SZENT-GYÖRGYI[7]) as well as in other metabolic processes (WEAVER and LARDY[8], URATA and GRANICK[9], GREEN and ELLIOTT[10], REEVES and AJL[11], JERZYKOWSKI et al.[12]).

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The Nature of DNA Synthesis by Isolated Nuclei from Cells of a Rat Tumour

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Summary. The DNA synthetic activity of nuclei isolated from a solid rat tumour was determined. The nuclei had DNA synthetic properties similar to nuclei from other sources but the time course of the reactions was different.

Recently[1-6] many workers have shown that nuclei isolated from mammalian cells contain a DNA synthesizing system which has features in common with that described for the membrane-DNA fragments obtained from bacteria[7-11]. These nuclei utilizing endogenous polymerases and chromatin, are capable of the continuation of DNA and RNA[2] synthesis in the presence of an adequate supply of precursors. Earlier workers (loc. cit) have investigated the DNA-synthetic properties of nuclei obtained from a wide range of mammalian cells and there is considerable evidence that it is possible to relate these properties to the status of the cells from which the nuclei were derived. The isolated nuclei provide a system by which the synthesis of macromolecules can be studied more directly than is possible in the whole cell system. For instance the effect of cytotoxic treatments on the nuclei can be determined without the involvement of the effect of the treatment on the cytoplasm.

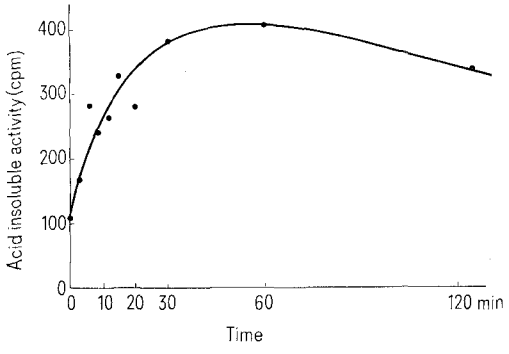

Fig. 1. The pattern of synthesis and degradation of new DNA as a function of time. Nuclei from rat fibrosarcoma RIB_{5c}.

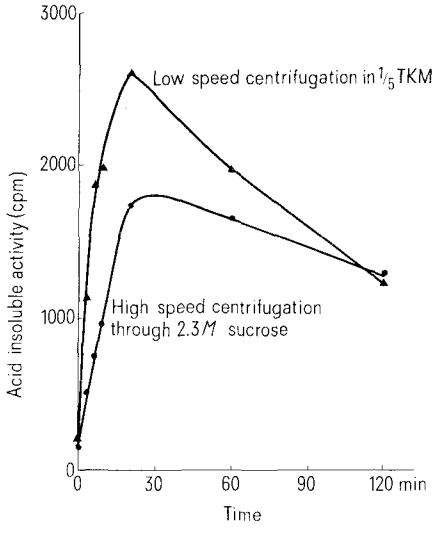

Fig. 2. Synthesis and degradation of new DNA by isolated nuclei. Final stages of isolation either through 1/5 TKM (▲); or through 2.3 *M* sucrose (●). The counts have been corrected for differences in initial concentrations of nuclei from RIB_{5c}.

To our knowledge cells from solid neoplasms have not been used as a source of nuclei. We have in our laboratory a series of transplantable rat tumours, of which, the gross responses to ionizing radiation have been studied in depth[12] and differences between these responses, not yet fully understood, may be due to differences in post-irradiation modifications of macromolecular synthesis. In this paper we present the results of experiments designed to establish the nature of the DNA-synthetic reaction in nuclei isolated from one of these rat tumours.

Isolation of nuclei from tumour tissues. The fibrosarcoma RIB_{5c} was excised from the flanks of an inbred strain of Wistar rat. They were immediately cooled in ice cold isotinic saline and all subsequent preparative procedures were at 0 °C. The tumour weighing 2–3 g was cut into small pieces and reduced to pulp by compression

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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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