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INHIBITION OF CELL DIVISION IN AMOEBAE: THE INCORPORATION OF TRITIATED PRECURSORS INTO *AMOEBA PROTEUS* AFTER THE INJECTION OF NON-HOMOLOGOUS CYTOPLASM

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SUMMARY

The injection of non-homologous cytoplasm into any strain of large free-living amoebae leads to a 60 % inhibition of division amongst recipient cells. When the post-microsomal supernatant fraction of *Amoeba discoides* was injected into *A. proteus*, this inhibition of division was as high as 95 %. The incorporation of tritiated precursors, either [^{3}H]uridine or ^{3}H-amino acids, into these inhibited amoebae was studied at various times after the injection of the inhibitory material using autoradiography.

When cells were grown in [^{3}H]uridine, autoradiographs indicated that RNA synthesis had ceased 2 days after the injection of non-homologous material. However, if [^{3}H]uridine was injected into the inhibited cells, some synthesis of RNA could be detected up to 4 days after the injection of inhibitor. These results suggested that uptake of [^{3}H]uridine was impaired and that one site of action of the inhibitory molecules was RNA synthesis for membrane components.

Experiments with a variety of ^{3}H-amino acids suggested that protein synthesis continued for at least 9 days after the injection of non-homologous cytoplasm, and that in these cells some informational RNA molecules were long-lived. There seemed to be accumulation of material containing [^{3}H]lysine in the nuclei of control cells taken at random from cultures, and this was seen in the nuclei of inhibited cells 1 day after injection. However, 2 days after the injection of inhibitor, no accumulation of [^{3}H]lysine-containing material was found in the nuclei.

INTRODUCTION

The microinjection of cytoplasm taken from one strain of large, free-living amoeba into another strain is followed by an incompatibility phenomenon, the inhibition of division amongst the recipient cells. When cytoplasm from *Amoeba discoides* was injected into *A. proteus*, 60% of the injected cells failed to divide. The molecules responsible for this inhibition have been shown to be in the post-microsomal supernatant fraction (Hawkins, 1969), such that when this fraction was injected into *A. proteus*, the inhibition was as high as 95 %. Jeon & Lorch (1970) have reported a similarly high inhibition of cell division, 93 %, when a supernatant fraction from *A. proteus* was injected into *A. discoides* and 60 % inhibition followed the injection of *A. proteus* cytoplasm into the multinucleate *Chaos carolinensis* (Yudin, 1973). The injection of homologous cytoplasm, and also a variety of materials including DNA, RNA and selected proteins resulted in at least 90 % division amongst the recipient cells, so that the inhibition of division is a property of the non-homologous cytoplasm.

Little is known of the molecules involved in this inhibition of division, or of their sites of action after injection into the recipient cell. Jeon & Lorch (1971) concluded, as a result of autoradiography, that [^{3}H]uridine incorporation decreased 4 h after the injection of *A. proteus* cytoplasm into *A. discoides* and that almost all RNA synthesis had ceased in these cells by this time.

We have studied the incorporation of tritiated precursors, either uridine or various amino acids, into inhibited *A. proteus* at various times after the injection of supernatant material taken from *A. discoides* using autoradiography. Although most of the cells were grown in the tritiated precursor, it was possible that these inhibited cells lacked the ability to transport materials across their cell membranes. In some experiments the tritiated precursor was injected into *A. proteus* at various times after they had been inhibited by the injection of *A. discoides* supernatant fraction.

MATERIALS AND METHODS

Living material

Amoeba proteus ($_{T1}$P) and *Amoeba discoides* ($_{T1}$D) were obtained originally from Taylor (Glasgow) in 1948, and since then have been kept in the Zoology Department, King's College, London. Both strains were grown in wheat grain cultures (Lorch & Danielli, 1953) in Chalkley's medium at 17 ± 1 °C. Large numbers of *A. discoides* used to prepare the post-microsomal supernatant fraction were grown after the method of Griffin (1960) and fed on *Tetrahymena pyriformis*.

Radioactive precursors

The radioactive precursors used were supplied by the Radiochemical Centre, Amersham. They were as follows: [5-^{3}H]uridine (29 Ci/mmol); L-[^{3}H]aspartic acid (178 mCi/mmol); DL-[2-^{3}H]glutamic acid (2·8 Ci/mmol); L-[4,5-^{3}H]leucine (38 Ci/mmol); DL-[4,5-^{3}H]lysine monohydrochloride (5·5 Ci/mmol); L-[5-^{3}H]proline (10Ci/mmol); L-[3-^{3}H]serine (6·3 Ci/mmol); L-[3,5-^{3}H]tyrosine (33 Ci/mmol).

Preparation of inhibited cells

Large numbers of *A. discoides* were bulked and lightly homogenized in sucrose-TKM buffer (0·24 M sucrose, 50 mM Tris-HCl, 25 mM KCl, 5 mM $MgCl_2$, pH 7·4) as described previously (Hawkins, 1969). The post-microsomal supernatant material was obtained after centrifugation of the homogenate for 2·5 h at 105 000 *g*. This supernatant material was stored in small aliquots at −20 °C until used. A small volume of supernatant material was injected into *A. proteus* using techniques described previously (Hawkins & Cole, 1965).

Each preparation of supernatant material was tested for its efficiency in inhibiting cell division by injecting 100 cells of *A. proteus* and growing these cells singly in watch glasses containing Chalkley's medium and food organisms (*Colpidium* sp. 500/ml). The percentage of cells dividing was determined, and was usually between 5–10 %.

Preparation of labelled cells

A. proteus, at various times after the injection of supernatant fraction of *A. discoides*, were grown for 5 h in either [^{3}H]uridine or one of the ^{3}H-amino acids, at a concentration of 40 μCi/ml in Chalkley's medium. After this time they were processed for autoradiography.

Control cells of *A. proteus* were either injected with sucrose-TKM buffer and grown in [^{3}H]uridine, or were placed directly in 40 μCi/ml [^{3}H]uridine for 5 h.

Some cells of *A. proteus* were injected with supernatant material from *A. discoides*, and at various times after this injection, [^{3}H]uridine, at 40 μCi/ml in sucrose-TKM buffer, was injected. They were then grown for 5 h in 40 μCi/ml [^{3}H]uridine in Chalkley's medium.

Control cells were injected with sucrose-TKM buffer, followed by [^{3}H]uridine and subsequently grown for 5 h in 40 μCi/ml [^{3}H]uridine in Chalkley's medium. All these cells were then processed for autoradiography.

Control cells for the amino acid experiments were grown in 40 μCi/ml of the appropriate precursor.

Preparation of autoradiographs

After growth in the tritiated precursor, cells were transferred to Chalkey's medium. Groups of cells were placed at one end of a clean glass slide and after they had attached to the substrate, the excess Chalkley's medium was removed. A coverslip with a drop of 70 % ethanol was used to squash the amoebae, flattening them on to the slide. After freezing on Cardice the coverslip was removed. Slides prepared in this way were placed for 15 min in 96 % ethanol, followed by 15 min in 5 % trichloroacetic acid and then two 15-min washes in 70 % ethanol. After this slides were placed in fresh 70 % ethanol at 4 °C and were left overnight. The slides were air-dried and dipped in a 1:1 Ilford K2:water emulsion containing a little gycerol (Rogers, 1967). After exposure to film for 3 weeks at 4 °C, the autoradiographs were developed in D-19 for 4 min, rinsed and fixed in Ilfofix for 8 min, rinsed and air-dried. When [^{3}H]serine was used as a precursor, the slides were exposed for 4 weeks, and a 7-week exposure was used for [^{3}H]aspartic and [^{3}H]glutamic acids.

After mounting in Euparol, the silver grains were counted using a Zeiss microscope and × 100 phase contrast. The size of the nucleus was noted (in squares of an eyepiece graticule, each 240 μm^2) and the number of grains in the nucleus, 3 × 10 squares of cytoplasm and 3 × 10 squares of background were counted. Taking into account the background, and the thin layer of cytoplasm over the nucleus, all grain counts were corrected to, and expressed as grain counts per square.

RESULTS

Experiments with [^{3}H]uridine

Growth for 5 h in [^{3}H]uridine was chosen for these experiments because it had been found that in this time the nuclei of most cells were well labelled (S. E. Hawkins, unpublished). Since very little cytoplasmic labelling was obtained in this time, only the nuclear grain count was considered. The grain count per nuclear square of control *A. proteus* is shown in Fig. 1. These control cells were either grown in 40 μCi/ml [^{3}H]-uridine for 5 h (Fig. 1A), or injected with sucrose-TKM buffer followed by growth in [^{3}H]uridine (Fig. 1B), or injected with sucrose-TKM buffer containing 40 μCi/ml [^{3}H]uridine followed by growth in [^{3}H]uridine (Fig. 1C). The results obtained from cells grown directly in precursor showed that although 38 % of these cells were incorporating uridine into acid-precipitable material at a rate leading to labelling of the nucleus greater than 16 grains per square, there was considerable variation among the remainder of the cells (Fig. 1A), including a group comprising 11 % of the cells with less than 4 grains per nuclear square. Injection of buffered sucrose led to an increase in this lower group to 37 % (Fig. 1B). However, when [^{3}H]uridine was injected into these cells, this lower group disappeared and there was a marked increase in nuclei with the higher grain count (64 %, Fig. 1C). It appeared that the process of microinjection led to some temporary mechanical damage to the cell membrane, and that this would need to be taken into account when considering the activity of cells immediately after the injection of supernatant material from *A. discoides*.

The results obtained when cells were grown in [^{3}H]uridine at different times after

the injection of the inhibitory material, namely from 0–1 h to 4 days, are shown in Fig.2. There was some decrease in incorporation of precursor amongst cells placed directly into uridine after the injection of supernatant material from *A. discoides*, but when placed in precursor 12–13 h after injection the pattern of labelling was more like that of control cells placed directly in [^{3}H]uridine (Fig. 1 A). This indicated that the decrease seen amongst 0–1 h cells was due to mechanical damage, rather than to

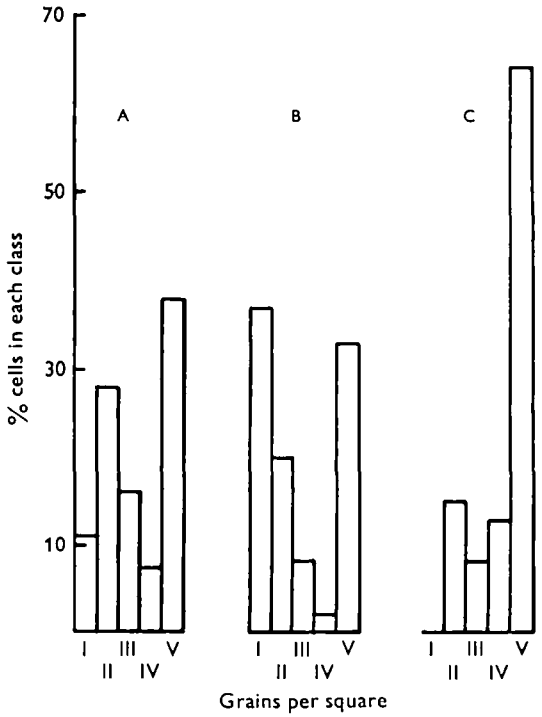

Fig. 1. Nuclear grain counts of control *A. proteus* grown in [^{3}H]uridine. A, cells grown in [^{3}H]uridine, 40 μCi/ml, for 5 h. B, cells injected with Tris-buffered sucrose and then grown in [^{3}H]uridine, 40 μCi/ml, for 5 h. C, cells injected with Tris-buffered sucrose containing 40 μCi/ml [^{3}H]uridine and then grown in [^{3}H]uridine, 40 μCi/ml, for 5 h. Grain counts per square of nucleus: class I = 0–3·9; II = 4–7·9; III = 8–11·9; IV = 12–15·9; V = 16 and over.

the direct action of the inhibitory molecules. After 23–24 h about 68 % of the injected cells showed a low grain count, 0–3·9 grains per nuclear square, but there still remained a number of more active cells. However 2 days after injection there was little incorporation of labelled precursor, and what little there was, was probably due to the small number of cells, about 10 % which always divide after the injection of inhibitory material. By 4 days after injection these had divided and 100 % of the injected cells showed little or no grain count in their nuclei (Fig. 2).

The possibility existed that these inhibited cells could no longer take up [^{3}H]-uridine from the growth medium. A series of cells, at various times after the injection of supernatant material from *A. discoides*, were injected with sucrose-TKM buffer containing 40 μCi/ml [^{3}H]uridine. They were then grown for 5 h in [^{3}H]uridine in

Chalkley's medium and processed for autoradiography. The results obtained can be seen in Fig. 3, and these showed that there was still incorporation of labelled precursor into acid-precipitable material 4 days after the initial injection of inhibitory material, 37 % of the cells having a nuclear grain count per square of 16 or more. However, there was a marked decrease in grain count by the sixth day after the initial injection. It appeared that the inhibited cells were still able to incorporate labelled precursor for a longer period than that indicated in the previous experiments, and that some damage to membrane-transporting systems had occurred by about 1 day after the injection of the supernatant fraction of *A. discoides*.

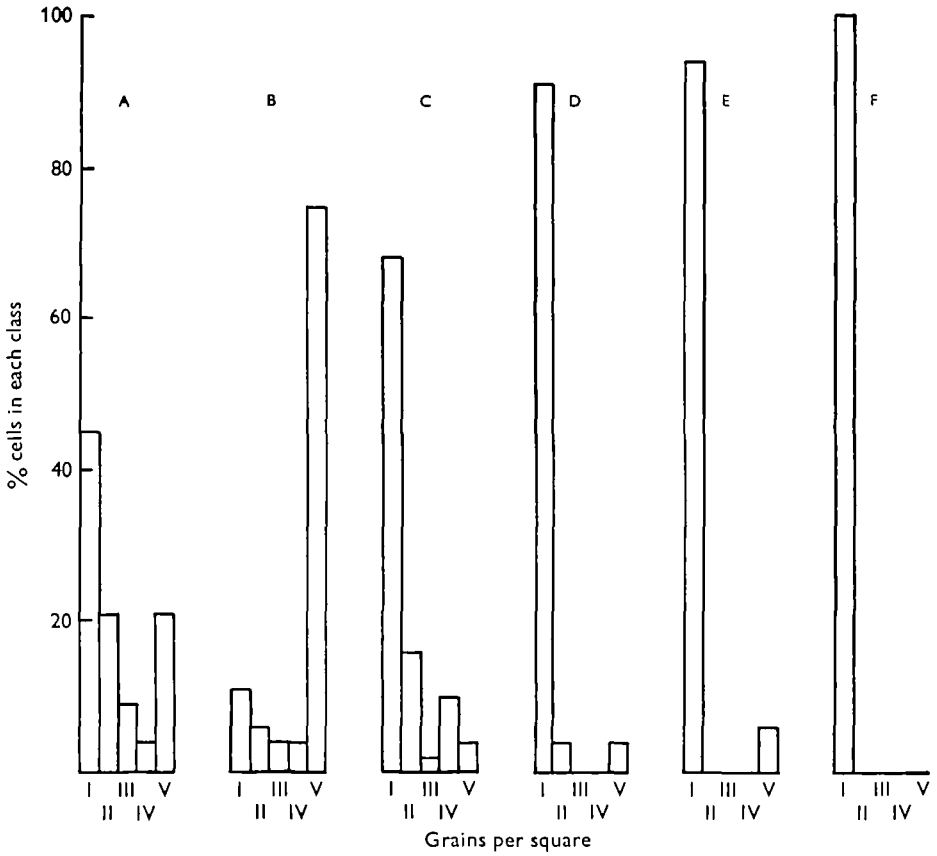

Fig. 2. Nuclear grain counts of *A. proteus* at various times after the injection of *A. discoides* supernatant. A, 0–1 h after injection; B, 12–13 h; C, 1 day after injection; D, 2 days; E, 3 days; F, 4 days. All cells were grown in [^{3}H]uridine, 40 μCi/ml, for 5 h, at time periods indicated. Grain counts per square of nucleus: class I = 0–3·9; II = 4–7·9; III = 8–11·9; IV = 12–15·9; V = 16 and over.

Experiments with ^{3}H-amino acids

The incorporation of ^{3}H-amino acids into normal and inhibited cells was examined by comparing the grain count per square of cytoplasm (240 μm^2) (Figs. 4–6). In normal cells the incorporation into acid-precipitable material varied greatly from one

amino acid to another. Thus when [^{3}H]leucine (Fig. 4A) and [^{3}H]lysine (Fig. 6A) were used, cells were heavily labelled, while [^{3}H]tyrosine (Fig. 5C), [^{3}H]proline (Fig. 5A) and [^{3}H]serine (Fig. 5C) were incorporated to a lesser extent. Aspartic and glutamic acids (Fig. 6C, E) showed rather little incorporation even though the exposure time used was 7 weeks.

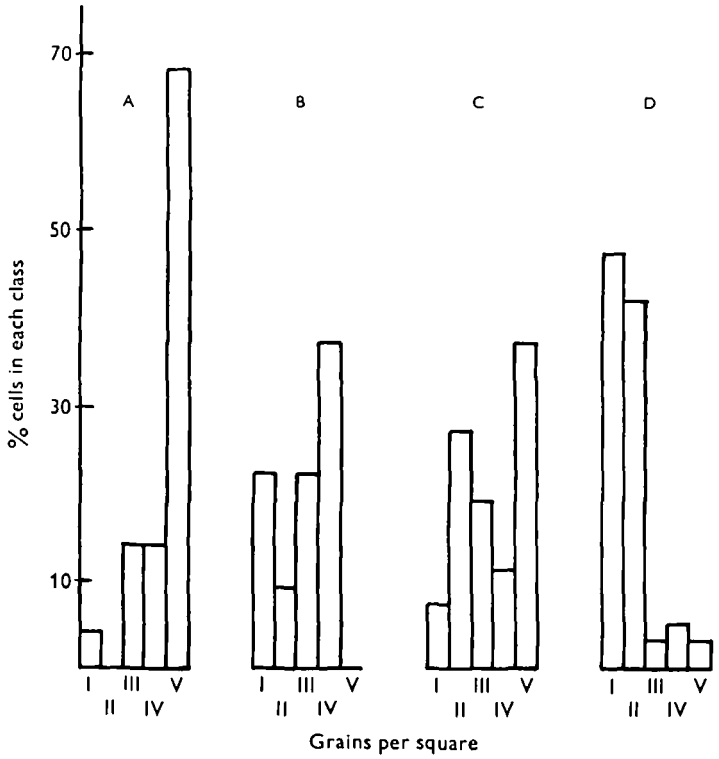

Fig. 3. Nuclear grain counts of *A. proteus* injected with *A. discoides* supernatant, and then at various times after this, injected with [^{3}H]uridine, 40 μCi/ml, before growth in [^{3}H]uridine, 40 μCi/ml, for 5 h. A, injection of [^{3}H]uridine 4 h after injection of inhibitor. B, [^{3}H]uridine 2 days after inhibitor; C, [^{3}H]uridine 4 days after inhibitor; D, [^{3}H]uridine 6 days after inhibitor. Grain counts per square of nucleus: I = 0–3·9; II = 4–7·9; III = 8–11·9; IV = 12–15·9; V = 16 and over.

Amoebae, at various times after the injection of inhibitory material, were grown in the labelled amino acids. The results of an experiment using [^{3}H]leucine from 1 to 7 days after injection are shown in Fig. 4. Despite a decrease in the grain count of the inhibited cells when compared with normal cells, where 71 % of the latter had grain counts of over 16 per square, there was still incorporation of [^{3}H]leucine. Even 7 days after the injection of inhibitory material over 50% of the injected cells had grain counts of 12 per square of cytoplasm or above. Similarly, there was incorporation of proline, tyrosine, serine (Fig. 5B, D, F) and lysine (Fig. 6B) several days after the injection of the inhibitory molecules.

When [^{3}H]aspartic and [^{3}H]glutamic acids were used as precursors, there was a low level of incorporation amongst the control cells. Cells examined 1 day after the

injection of inhibitor showed even less incorporation, most cells giving grain counts of less than 4 per square of cytoplasm (Fig. 6D, F).

The grain count per square of nucleus for the majority of the amino acids used in these experiments was low. However, when [^{3}H]lysine was used, a substantial grain count was obtained in control cells. Fig. 7 shows the proportion of the nuclear grain count to the cytoplasmic count per square in a control group of cells in [^{3}H]lysine,

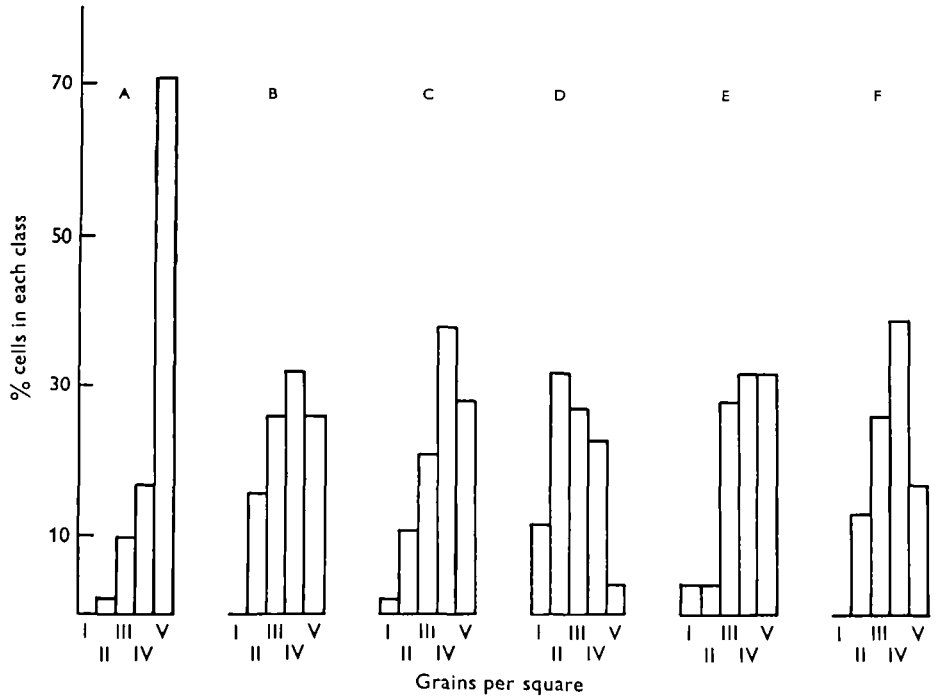

Fig. 4. Cytoplasmic grain counts of control and inhibited *A. proteus* grown in [^{3}H]leucine, 40 μCi/ml, for 5 h. A, control cells; B, cells 1 day after injection of inhibitor; C, cells 2 days after inhibitor; D, cells 4 days after inhibitor; E, cells 6 days after inhibitor; F, cells 7 days after the injection of inhibitor. Grain counts per square of cytoplasm: I = 0–3·9; II = 4–7·9; III = 8–11·9; IV = 12–15·9; V = 16 and over.

and the same ratio in cells 1 and 2 days after the injection of inhibitory material. Although in control cells and in cells 1 day after injection there was, proportionally, a large nuclear grain count, by 2 days after injection (Fig. 7C) the injected cells had only a low nuclear count. It appeared that transport of lysine-rich molecules had greatly decreased 2 days after the injection of non-homologous cytoplasm. On this occasion transport of precursor into the cell was not involved, since the cytoplasmic grain count of cells 3 days after injection was as high as that of control cells (Fig. 6A, B).

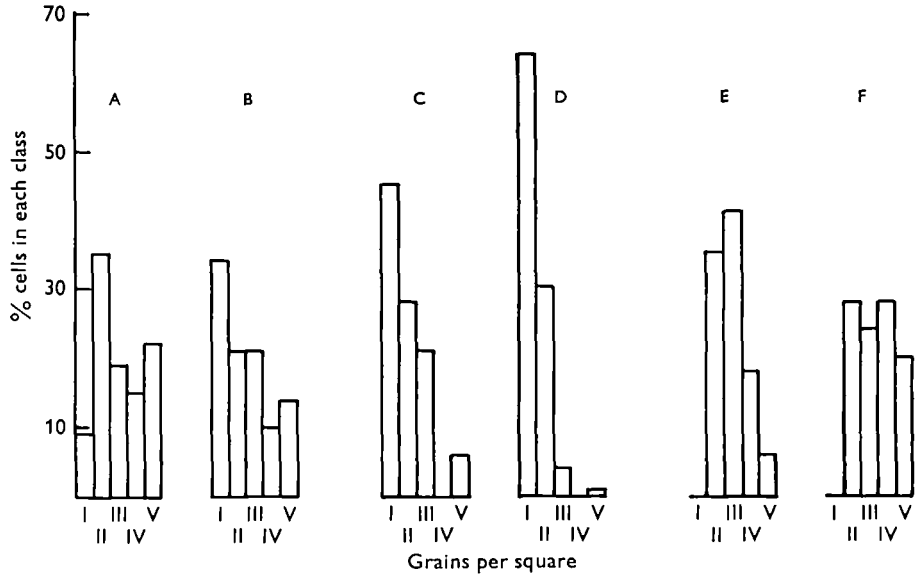

Fig. 5. Cytoplasmic grain counts of control and inhibited *A. proteus* grown in either [^{3}H]proline, [^{3}H]tyrosine or [^{3}H]serine, each at 40 μCi/ml, for 5 h. A, control cells grown in proline; B, cells grown in proline 9 days after injection of inhibitor; C, control cells grown in tyrosine; D, cells grown in tyrosine 5 days after injection of inhibitor; E, control cells grown in serine; F, cells grown in serine 3 days after injection of inhibitor. Grain counts per square of cytoplasm: I = 0–3·9; II = 4–7·9; III = 8–11·9; IV = 12–15·9; V = 16 and over.

DISCUSSION

In order to study the effects of the inhibitory molecules on RNA synthesis in amoebae, autoradiography was selected as a means of examining single cells. Although restrictive in terms of the number of cells it was possible to observe, this method did allow some assessment of individual variability, a parameter lost in many other types of experiment. It was necessary to select a labelling time which would give some idea of the presence or absence of incorporation and also be restrictive enough to give some indication of the time taken for inhibitory activity. The amount of radioactivity accumulating in the total RNA during this labelling period will reflect both synthesis and degradation of the several RNA species in the cell and will be influenced by the relationship between radioactive precursor and the cellular precursor of RNA. Earlier observations indicated that in amoebae the appearance of radioactivity in nuclei was considerably slower than that seen in most eukaryotic cells, and that 5 h was the minimum time required to see well labelled nuclei (S. E. Hawkins, unpublished). However, even after 5 h, very few radioactive molecules had been transferred to the cytoplasm. This may reflect the absence of heterogeneous RNA (HnRNA) in amoebae as proposed by Prescott, Stevens & Lauth (1971), since in most eukaryotes HnRNA forms a pool of material which is most rapidly turned over. In addition, no information is available as to the number of radioactive uridine molecules which enter the cell and are subsequently phosphorylated by kinases to produce ultimately

uridine triphosphate (UTP). Furthermore the size of the pre-existing UTP pool cannot be measured directly due to insufficient material available in cells (Cooper, 1972).

Thus autoradiographs reflected the acid-precipitable material present in amoeba nuclei after a 5-h labelling period and enabled nuclei from injected, inhibited cells to be compared with those of normal cells at varying times after injection. There was

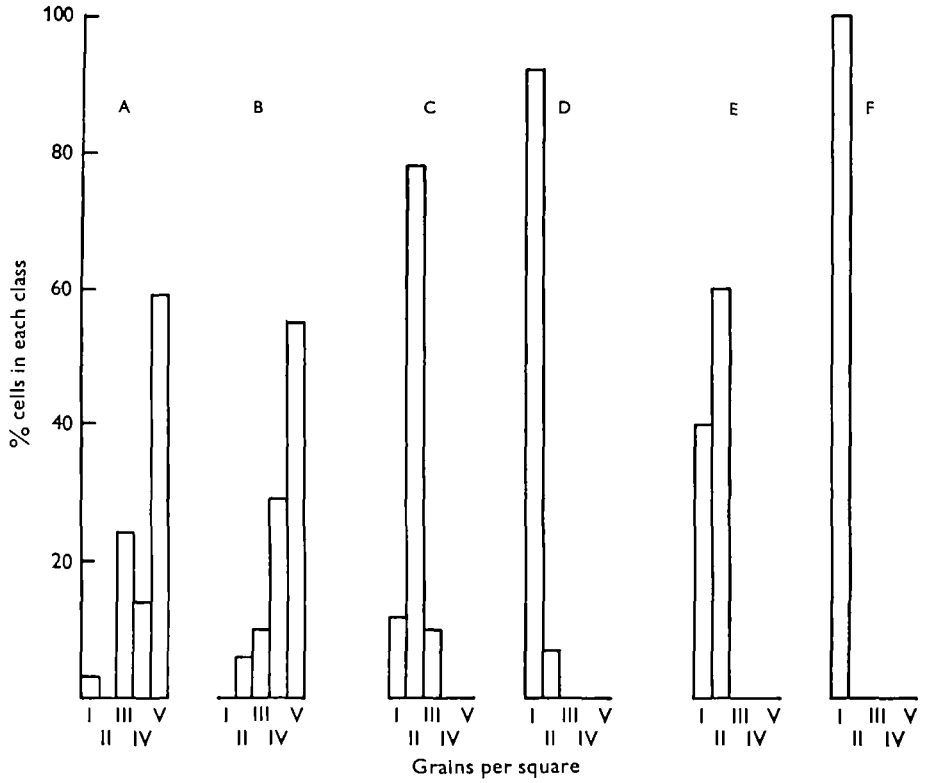

Fig. 6. Cytoplasmic grain counts of control and inhibited *A. proteus* grown in either [^{3}H]lysine, [^{3}H]aspartic acid or [^{3}H]glutamic acid, each at 40 μCi/ml, for 5 h. A, control cells grown in lysine; B, cells grown in lysine 3 days after injection of inhibitor; C, control cells grown in aspartic acid; D, cells grown in aspartic acid 1 day after injection of inhibitor; E, control cells grown in glutamic acid; F, cells grown in glutamic acid 1 day after injection of inhibitor; grain counts per square of cytoplasm: I = 0–3·9; II = 4–7·9; III = 8–11·9; IV = 12–15·9; V = 16 and over.

some decrease in the incorporation of [^{3}H]uridine in cells from 0–1 h after the injection of supernatant material from *A. discoides*. This was probably due to mechanical damage, since control cells injected with Tris-buffered sucrose also showed decreased activity, unless [^{3}H]uridine was injected at the same time. Cells 12–13 h after injection of inhibitory material showed incorporation of precursor which compared favourably with that seen in control cells. It was not until 23–24 h after injection that any real difference between control and inhibited cells was seen, with an increased proportion

of apparently inactive cells. About 2 days after the injection of inhibitory material, the cells appeared no longer to incorporate labelled precursor.

These results do not support the results of Jeon & Lorch (1971) who suggested that all RNA synthesis, as measured by autoradiography, had stopped by 4 h after the injection of inhibitory material. However, their cells were grown in [^{3}H]uridine for 22 h, too long to be considered as a 'pulse', and they were unable to assess activity at

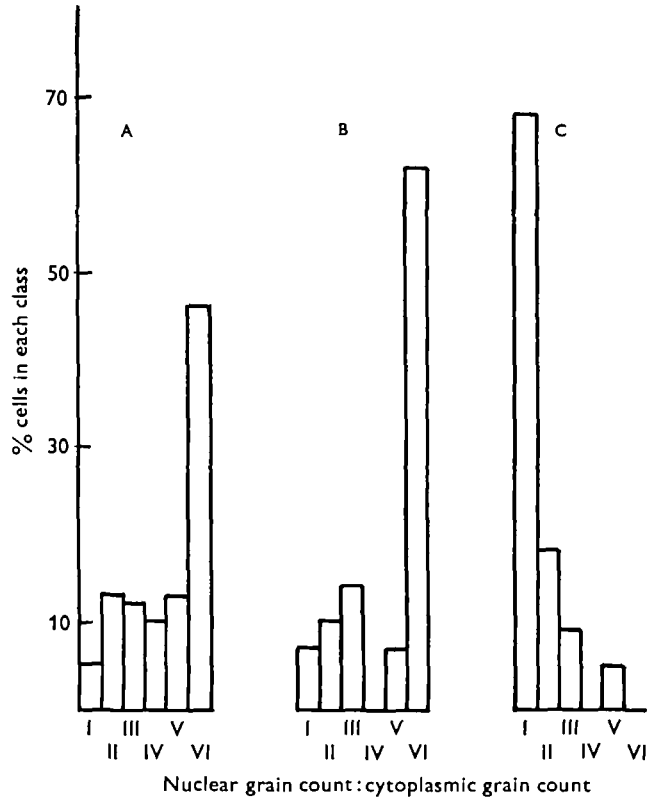

Fig. 7. The ratio of nuclear to cytoplasmic grain count of control and inhibited *A. proteus* grown in [^{3}H]lysine, 40 μCi/ml, for 5 h. A, control cells; B, cells grown in lysine 1 day after injection of *A. discoides* supernatant; C, cells grown in lysine 2 days after injection of *A. discoides* supernatant. Nuclear grain count per square : cytoplasmic grain count per square; I = 0·0–0·19; II = 0·2–0·39; III = 0·4–0·59; IV = 0·6–0·79; V = 0·8–0·99; VI = 1·0 and above.

shorter time periods. Their results, which are comparable with those we obtained 23–24 h after injection, indicated that there was some labelling in inhibited cells after this time, although grain counts were not given.

However, observation of cells grown directly in [^{3}H]uridine gave misleading results since experiments where [^{3}H]uridine was injected into inhibited cells indicated that for at least *4 days* after the injection of inhibitory material, these cells were able to synthesize some RNA. After 6 days there was again little incorporation of precursor even when it was injected. Thus if [^{3}H]uridine was available in these cells, synthesis

of RNA continued up to 4 days after the injection of supernatant material from *A. discoides*.

Little is known about the mechanism of uridine transport into *A. proteus*, or indeed of the amount of recycling of pre-existing uridine from other sources, e.g. food organisms. In the lymphocyte there seem to be specific sites for uridine uptake in the plasma membrane (Peters & Hausen, 1971), and under certain circumstances, for instance phytohaemagglutinin activation, the number of sites for uridine transport increases. The inability of the inhibited amoebae to take up uridine 1–2 days after the initial injection could be due to a decreased number of binding sites, reflecting changes in the plasma membrane. There is some evidence that information for membrane turnover is short-lived in *A. proteus*. Experiments using nuclear transfer techniques and inhibitors of RNA synthesis by Flickinger (1971) showed that the Golgi apparatus, which is thought to be involved in the production of material destined for the cell surface, was dependent on nuclear RNA synthesis. Saunders & Bell (1970) have indicated that newly divided amoebae do not show pinocytosis, a process requiring membrane turnover, for some time after division. Indeed after a cycle of pinocytosis amoebae require at least 4 h to 'recover' before commencing another cycle. If normal membrane function, both of internal membranes and the plasma membrane, does rely on newly synthesized RNA, then the inability of amoebae 1 day after injection with non-homologous cytoplasm to transport uridine across their membranes may indicate that some informational RNA necessary for normal membrane function was no longer being made. Since radioactive RNA molecules were still observed between 2–4 days after injection when [^{3}H]uridine was supplied directly, other effects for instance on informational RNA for kinases or other proteins appeared less immediate.

The results of the experiments using labelled amino acids showed considerable incorporation into acid-precipitable material, and indicated that either some informational RNA was made several days after the injection of inhibitory material, or that a proportion of this RNA, as well as ribosomal RNA, was long-lived and, for some time, stable. That some stable informational RNA molecules exist in these amoebae is apparent from the results of Ord (1968*a*) who showed that it was possible to obtain 70% survival amongst cells re-nucleated up to 6 days after enucleation. Also Mazia & Prescott (1955) proposed that some protein synthesis, although at a reduced rate, occurred in *A. proteus* for at least 3 days after enucleation. Continued protein synthesis in the presence of impaired RNA synthesis occurred in inhibited amoebae, but whether these proteins were functionally normal could not be determined in our experiments. Certainly inhibited cells increased in size for several days before signs of deterioration could be seen.

The results of experiments using aspartic and glutamic acids appeared to differ from those using other amino acids. However, there was rather little incorporation of these precursors in the control cells. One day after injection there was virtually no incorporation. Friz (1970) has shown that both these amino acids are present in quite large amounts in amoebae, so that the low level of incorporation in control cells was not due to rarity. The decreased incorporation in the inhibited cells could have been

due either to the lack of transport of these 2 amino acids into the cell, or, as both these amino acids are present in substantial amounts in the cell membrane (Allen & Winzler, 1973), to a decrease in incorporation of these precursors into membrane proteins. This problem might be resolved by the injection of aspartic or glutamic acid into the inhibited cells.

The experiments using [^{3}H]lysine indicated that there was some accumulation of lysine-rich material in the nuclei of control cells, and in the nuclei of cells 1 day after the injection of inhibitory material. This accumulation had disappeared in amoebae placed in [^{3}H]lysine 2 days after injection. Robbins & Borun (1967) showed that the synthesis of histones in HeLa cells is closely correlated with that of DNA synthesis. Amoebae synthesize DNA directly after division and for the following 8 h (Ord, 1968*b*), the remaining 48 h being spent in G_2. In cells taken at random from a culture most might have been expected to have already replicated their DNA and their histones, if the HeLa cell model is applicable to amoebae. Thus the [^{3}H]lysine may be incorporated into some other protein fraction which migrates into the nucleus. Of the proteins in *A. proteus* there is a class known to shuttle back and forth between nucleus and cytoplasm. The only one studied in detail, however, is acidic in nature (Jelinek & Goldstein, 1973). This does not preclude migration of protein with a large number of basic residues throughout the cell cycle into the nucleus, as seen in control cells. Whatever the nature or function of this protein, or proteins, 2 days after the injection of inhibitory material no accumulation occurred in the nuclei of the inhibited amoebae, although incorporation still could be demonstrated in the cytoplasm.

The nuclear membrane is known to show selectivity of uptake towards certain proteins (Gurdon, 1974), and these results might indicate early impairment of membrane function, in this instance the nuclear membrane, and reflecting the inability of the damaged membrane to transport lysine-labelled molecules into the nucleus.

The inhibition of cell division in *A. proteus* following the injection of supernatant material from *A. discoides* was characterized by a decrease in the incorporation of [^{3}H]uridine. We propose that the first site of action of the inhibitory molecules may be informational RNA synthesis, and that this is followed by the decreased synthesis of other RNA species. Among these informational molecules those involved in the maintenance of the Golgi apparatus and directly or indirectly in the direction of membrane protein synthesis, whether internal or external membranes, may have a relatively short lifetime. Other informational molecules have a much longer lifetime as evidenced by the demonstration of incorporation of ^{3}H-amino acids up to 9 days after the initial injection. Not only does this system offer a means of investigating the control of cell division in amoebae, it also offers a means of determining the lifetime of informational RNA molecules in these eukaryotic cells.

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