only an autoradiographic approach to the problem of penetration is possible. With the second, in which Zajdela ascitic hepatoma cells are incubated in the presence of labelled NHCP, the labelling distribution inside the cells can be studied by means of a biochemical extraction method.

Materials and methods. Nuclei from hepatic cells of adult *Pleurodeles* and from Zajdela ascitic hepatoma cells are isolated as described in [16]. The chromatin and then the NHCP are isolated according to a modification by KRUH [14] of WANG's method [17]. NHCP can be kept frozen at −80 °C in 5×10^{-2} *M tris* HCl (pH 8.5). All operations are performed at 4 °C.

NHCP labelling. ^{125}I labelling of NHCP is performed by an adaptation of the enzymatic method (use of solid state bovine lactoperoxidase) of David [18]. Free iodine is separated from labelled NHCP by means of chromatography on a (15 × 0.9 cm) G 15 Sephadex column. All operations, except labelling, are performed at 4 °C. The exposed cells are embryonic cells, obtained from medullary plate and the chordomesodermal region of *Pleurodeles waltlii* embryos at the neurula stage. After disaggregation the isolated cells are cultivated according the technique of Duprat [19] in plastic micro-chambers. The above cells are treated with Barth's solution, without bovine serum albumin (BSA) but with labelled NHCP extracted from hepatic *Pleurodeles* (homospecific proteins) cells or from Zajdela ascitic hepatoma (heterospecific proteins) cells, with an active protein concentration of 50 µg/ml culture medium [15]. After the period of treatment, they are washed with Barth's solution, fixed in situ for 24 h with 5% formol in Sorensen's 0.1 *M* phosphate buffer at pH 7.4. The cells are embedded in epon. Sections about 1500 Å thick are placed for optical autoradiography on slides (previously covered with gelatin and dessicated) and then coated with a 50 µm Ilford L4 emulsion layer. After 1 to 3 weeks' exposure (at 4 °C in a dehydrated atmosphere), specimens are developed for 20 min at 14 °C.

Zajdela ascitic hepatoma cell labelling. Cells cultivated in 5 ml flasks or in spinners in Glasgow medium are incubated at 37 °C for 6 h in the presence of ^{125}INa labelled NHCP (50 µg/ml medium). The nuclei are extracted as described in [16]. Cytoplasmic and nuclear fractions are isolated and their radioactivity determined.

Results and discussion. The NHCP labelling technique using iodination allows specific activities between 0.5 and 2.5×10^6 dpm/µg to be obtained. The labelling is stable in our incubation conditions for periods at least as long as those of our penetration studies. In any case, the radioactivity precipitated by trichloracetic acid does not decrease over this period when maintained at 4 °C (taking into consideration the decrease in ^{125}INa radioactivity). Our modified iodination method does not change NHCP biological activity, since homospecific labelled NHCP added to a culture medium at a concentration of 50 µg/ml was previously found to inhibit morphological differentiation of neurons and alter muscular cell differentiation [15]. From autoradiography carried out on sections 1500 Å thick, it is seen that almost all the nuclei are hardly labelled at all, unlike the cytoplasm which is very significantly labelled (Figure). Even after 16 h incubation of cells in the presence of NHCP, no significant nuclear labelling is observed. This agrees with the results of the study of ^{125}I labelled NHCP ascitic (Zajdela) hepatoma penetration in the cells of the same tissue. Almost all the radioactivity is localized in the cytoplasmic fraction (Table) as cellular fractionation and determination of the radioactivity of the different fractions show. Although preliminary results lead us to think that most of the proteins are labelled with high specific activity, we cannot exclude the hypothesis that some of them are scarcely labelled at all and thus escape monitoring in autoradiography. In addition, using the autoradiographic technique on whole cells treated and fixed on culture chambers without detaching them from their support, we cannot conclude that there is a difference in labelling between the various cellular types, whereas on the biological tests, only the neurons are sensitive to NHCP action [15]. Furthermore, heterospecific labelled NHCP penetration showed a cytoplasmic labelling as great as labelling obtained with homospecific NHCP; but it has previously been shown that heterospecific NHCP does not act on morphological cellular differentiation. These results thus give evidence within the limits of our autoradiographic technique for a) penetration and concentration of proteins at the cytoplasmic level and not at the nuclear level, which raises the question of how they act [15]. b) Homospecific and heterospecific proteins penetrate the cells equally well, although the heterospecific ones have no action on cell differentiation, unlike the homospecific ones.

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Cell Types Originating from Kidney Explants of Young and Old Mice

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Summary. Explants from young and old mouse kidneys give rise to two different cell types when placed in organ culture dishes. The two cell types differ in morphology and ability to grow in vitro. Explants from young mice give rise to one predominant cell type; those from old mice give rise to another. Our data supports the mosaic theory of aging.

Since the initial reports by CARREL and BURROWS [1] that cells emigrate more rapidly from explants taken from young animals than those taken from older animals, this phenomenon, referred to as 'latency', has been studied in the hope of gaining insights into the general problem of organismal and cellular aging [2–4].

EPHRUSSI and LACASSAGNE [5] noted that different cell types emigrated from explants taken from young and old animals, as did SOUKUPOVA et al. [6]. A majority of these studies have been qualitative, made on explants cultured in plasma clots or hanging drops, and generally only continued for a few hours or days. Our experiments

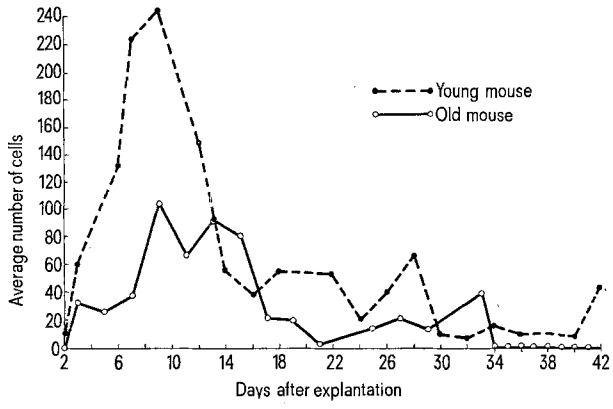

Fig. 1. Abscissa: Time (days); ordinate: average number of cells growing out of explants. ·- - - -·, young mouse; ▲— — —▲, old mouse.

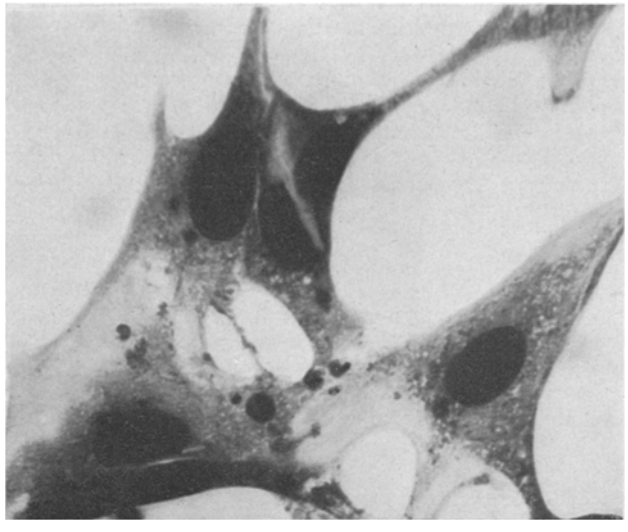

Fig. 2. 'A' type cells ~×600.

Fig. 3. 'B' type cells ~×600.

have been designed to obtain qualitative and quantitative data on the number and type of cells emigrating from explants taken from mouse kidneys, and to study the growth capacity of these cells in vitro.

Young (4-week) and older (8- to 9-month) male, I.C.R. strain mice, were killed by cervical dislocation. The left kidney was removed, the capsule peeled off and the cortex minced into 2 to 3 mm fragments in Eagle's minimal essential medium (MEM) with Hanks' salts, 10% heat-inactivated fetal calf serum, 2,000 m*M* glutamine/l, and antibiotics (5,000 units of penicillin and 5,000 µg streptomycin/ml) at 37 °C. In some experiments, 2 kidneys from mice of the same age were pooled.

Three fragments were placed on stainless-steel grids in the center of Falcon organ culture dishes (Falcon No. 3010); the well contained a cover slip and sufficient medium as described above to allow a meniscus of medium to cover the explants. The outer well of the dish contained sterile water, and the whole assembly was incubated at 37 °C in an atmosphere containing 5% CO_2 and 95% air and saturated with water.

At 24-hour intervals the grid and explants were placed in a new assembly. The cells that had collected on the cover slip were counted, then fixed in absolute methanol and stained with Giemsa. The experiments were terminated when cells ceased to emigrate from explants.

The data in Figure 1 demonstrate that more cells emigrate from young mouse kidney explants than from similar explants taken from older mice. Both sets of explants seem to achieve maximal emigration between the 9th and 10th days after explantation. Cellular emigration from the explants taken from young mice continues for at least 8 days longer than from the explants taken from old mice.

The emigrating cells have two distinct morphologies. The first or 'A' type cells (Figure 2) are highly cytoplasmic, have undulating borders, and generally contain

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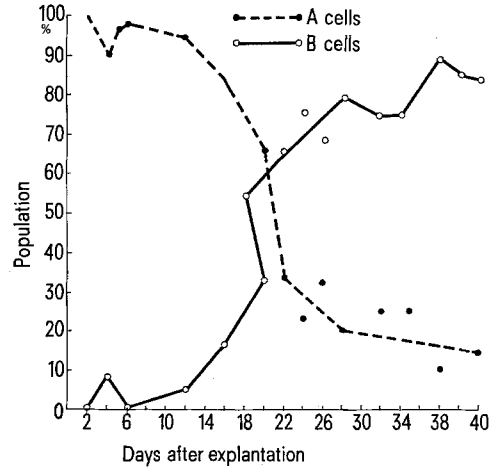

Fig. 4. Abscissa: Time (days); ordinate: average number of cells growing out of explants of young mouse kidney. - - - -, A cells; ▲— — —▲, B cells.

only 1 nucleolus. These cells have an average length of 13.4 ± 1.8 µm and were 4.8 ± 0.2 µm wide, and their nuclei, an average diameter of 3.2 ± 1.2 µm. The second or 'B' type cells (Figure 3) are considerably smaller than the A cells, and their nuclei, often multi-nucleolate, have an average length of 5.8 ± 1.0 µm and are 2.4 ± 0.4 µm wide.

The relative distribution of the two cell types emigrating from young and old mouse kidney explants is shown in Figures 4 and 5. It can be seen that A cells make up 80–90% of the emigrating population from young explants for the first 16 days, after which they decline and B cells rapidly become the dominant, but never the only, cell type.

In explants taken from old mouse kidneys the A cells never are the predominant cell type, only reaching 50% of the total population after 8 days, and declining thereafter. The B cells are dominant from the start and continue to emigrante from the explant after A cells no longer emigrate.

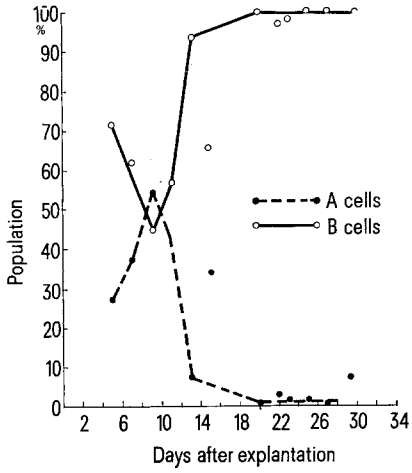

Fig. 5. Abscissa: Time (days); ordinate: average number of cells growing out of explants of old mouse kidney. - - - -, A cells; ▲——▲, B cells.

When subcultured in the same medium in which the explants were placed, the A cells were capable of a least 7 population doublings, whereas the B type cells could only double 2, or at most 3, times in this medium. These cells maintained their singular morphologies during growth; we did not observe any morphological instability in either cell isolates.

Our observations lend support to the suggestion first made by SOUKUPOVA and HOLECKOVA[7] that '...explanted tissue looks like a mosaic of more or less active and inactive parts, and the percentage of inactive parts increases with increasing age of the donor of the organ'.

The differences in the in vitro growth ability of the A and B cells supports the hypothesis that the mosaic refers to a combination of cells with low and high growth potential, consonant with the hypothesis expounded by HAYFLICK[8] for the cellular basis for aging.

Before accepting this hypothesis in toto one should remember that the medium used and the ratio between the number of cells in the inoculum and the volume of medium has a powerful effect on the ability of cells to grow in vitro[9]. Furthermore, some cell types have been shown to be unable to survive in the absence of other cell types[10]. Our data are similar to those reported by FRANKS and COOPER[11] who reported that two predominant cell types were found in cell lines originating from human lung embryos.

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Untersuchungen über stabile und während der frühen Quellungsstadien neu synthetisierte Poly(A)-RNA von *Agrostemma*-Samen

Studies on Stable and During Early Imbibition Phases Synthesized Poly(A)-RNA of *Agrostemma githago* Embryos

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Summary. RNA isolated from dry embryos of *Agrostemma githago* seeds contains poly(A)-sequences, but in very small amounts. In the early phase of imbibition, an intensive synthesis of poly(A)-containing RNA is brought about. The importance of this synthesis of poly(A)-RNA is discussed.

Die Natur der frühen RNA-Synthese quellender Samen und ihre Bedeutung für die Samenkeimung ist umstritten[2]. Nach Befunden mehrerer Autoren setzt die RNA-Synthese erst in der späten Quellungsphase, möglicherweise erst kurz vor dem Radiculadurchbruch ein, so dass die Proteine in diesem Zeitraum ausschliesslich an langlebiger mRNA gebildet werden dürften[3–6]. In ungequollenen Samen sind solche stabilen mRNA, die bereits während der Embryogenese transkribiert wurden[7], eindeutig nachgewiesen[8–10]. Unmittelbar nach Quellungsbeginn wurden dagegen beim Weizen[11–13], Roggen[14], MAIS[15] oder bei *Phaseolus angularis*[16] RNA-Synthesen gemessen. Über die Natur der frühen RNA-Synthese gibt es keine einheitliche Auffassung. CHEN et al.[11] haben bei Weizenembryonen in den ersten Quellungsstunden PrärRNA- und rRNA-, aber keine mRNA-Synthesen gefunden. Auch nach ihren Vorstellungen soll die unmittelbar mit Quellungsbeginn einsetzende Proteinbiosynthese[17] durch langlebige mRNA gesteuert werden. Andererseits wurden von einigen Arbeitsgruppen auch Neusynthesen von Hn- bzw. mRNA während der frühen Quellungsstadien von Samen wahrscheinlich gemacht[12,15,16].