

single strain of micro-organism(17).

Summary. A rapid agar slide method for localization and visualization of hyaluronate lyase (hyaluronidase) has been described. This procedure is based on enzymophoretic techniques developed for localization of mammalian enzymes following agar gel electrophoresis. Differences in electrophoretic mobility between staphylococcal, streptococcal and bovine testicular hyaluronidase have been demonstrated. The use of enzymophoresis in agar for study of bacterial enzyme systems has been discussed as a valuable procedure for enzyme analysis during purification and characterization studies.

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Reutilization of Presumably Degraded Radioactive Label from a Chromosomal Fraction of Homologous and Heterologous Cells. (32064)

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Several authors have reported evidence for reutilization of DNA by cells in which it did not originate(1-7). Borenfreund and Bendich (1) labeled purified DNA, suspended it with HeLa cells and later recovered the label from HeLa DNA. Bensch *et al*(7) combined pure DNA with gelatin and studied its phagocytosis and digestion by cultured fibroblasts.

The purpose of this research was to develop the necessary methods and study the uptake of labeled DNA from isolated whole chromosomes by dividing cells of the same and different species.

Materials and methods. Labeling, cell synchronization and preparation of a chromosome suspension. The cell line employed was Chinese hamster fibroblast strain V37/A clone F, generously provided by Dr. J. Mendelsohn

and Dr. N. P. Salzman. The cells were grown as monolayer cultures in modified Eagle's medium, according to Robbins and Marcus(8) supplemented with 10% fetal calf serum. Tritiated thymidine, 0.5 $\mu\text{c}/\text{cc}$ (specific activity 14.4 curies/millimole) was added to each of 6 large Blake culture bottles freshly planted with 4×10^6 Chinese hamster fibroblasts. Five hours later, Colcemid was added to a final concentration of 0.02 gamma/cc. The cultures were harvested at 20 hours, or 15 hours after Colcemid was added, when 70-80% of the cells are in metaphase. At this time the medium was substituted with prewarmed 1% sodium citrate solution (37°C). The bottles were then inverted with the cell layer uppermost and shaken forcefully several times to dislodge the swollen metaphase cells

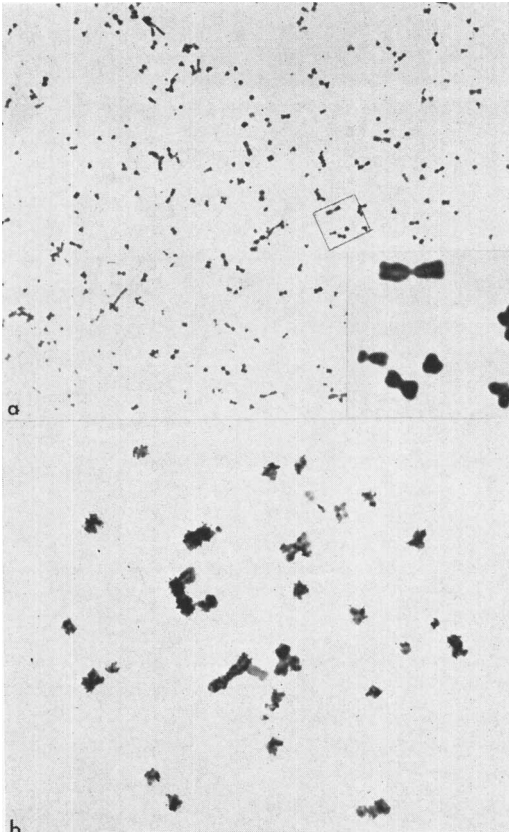


FIG. 1. Preparations of suspensions of mitotic chromosomes from synchronized Chinese hamster cultures, (a) without tritiated thymidine, (b) labeled with H^3Tdr .

(8). The cell suspension was poured into a 40 ml centrifuge tube, allowed to stand 10 minutes, and then centrifuged for 10 minutes at 800 rpm. The cells were resuspended in 0.1 M citric acid and the cell membranes were broken by repeatedly drawing and expelling the suspension through an 18 gauge spinal needle attached to a 20 cc syringe. Nucleated cells were removed by centrifugation at 500 rpm for 5 minutes and the chromosomes were collected from the supernatant by centrifugation at 3,000 rpm for 10 minutes. The button of chromosomes was washed twice with normal saline buffered at pH 7.0 with phosphate. The chromosomes were then resuspended in 1 cc normal saline for use. Chromosome preparations made from this suspension show very few interphase nuclei (<10 /preparation) (Fig. 1a, b).

Exposure of growing cells to labeled chromosomes. The above described suspension of chromosomes was used in 4 ways: (a) one bottle of freshly planted hamster monolayer cells ($1 \times 10^6/cc$) and one bottle of a suspension of human leucocytes ($1 \times 10^6/cc$) cultured for 48 hours, received 0.2 cc of the suspension of labeled chromosomes. Each bottle contained 12 cc of medium; (b) a second pair of culture bottles, prepared as in (a) differed only by addition of unlabeled thymidine at a final concentration of $5.04 \times 10^{-4}mg/cc$, a quantity which is about 10 times that of the labeled thymidine in the chromosome. This was estimated from the radioactivity in the chromosomes with the assumption that the specific activity of thymidine in the chromosomal DNA was equal to that of the radioactive thymidine used to label the chromosomes. (c) A culture bottle containing only the medium with calf serum and 0.10 cc of the chromosome suspension. Cells were not present. (d) A 0.10 cc sample of the suspension was used in the scintillation counter to determine the activity of H^3DNA .

Demonstration of incorporated tritium. As in preparing the original chromosome fraction, Colcemid was added to the cultures 5 hours after the labeled material was added. Both human leucocytes and Chinese hamster cells were harvested 10 or 20 hours later by the method of Rothfels and Siminovitch(9). Some of the harvested cells were used for cytological preparations and for autoradiography on AR-10 stripping film exposed for a week. On gross examination at least, no extraneous chromosomes were present nor were any abnormal chromosomal structures observed. Another portion of the cells was washed 3 times with cold isotonic saline. The cell pellet was then digested with 0.5 ml of a strong base in toluene (NCS reagent, Nuclear-Chicago). When digestion was complete, the material was quantitatively transferred to scintillation vials containing toluene scintillation liquid and the radioactivity counted in a tricarbol scintillation spectrometer. Correction for quenching was made by use of an external standard.

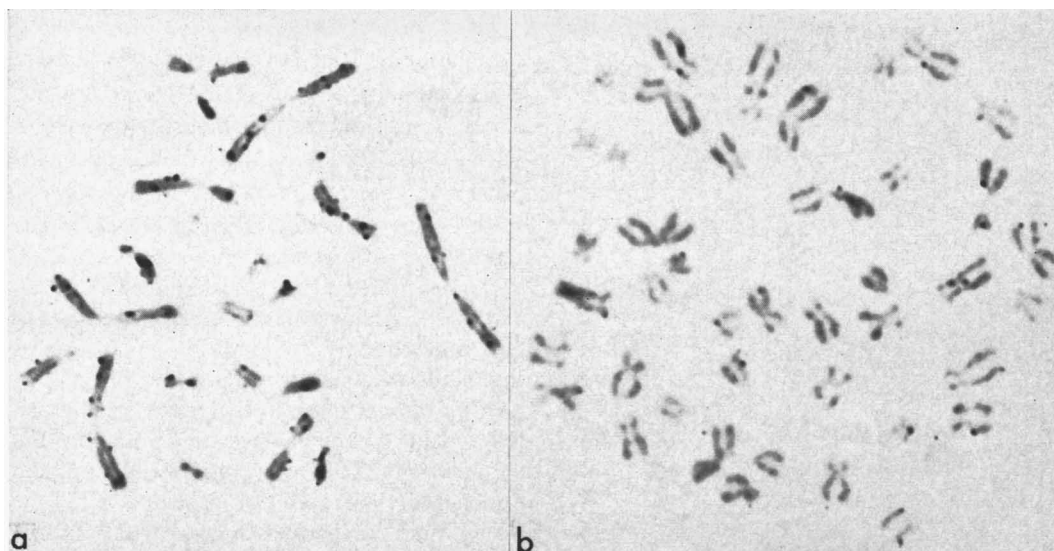


FIG. 2. Metaphase cells after autoradiography from (a) a Chinese hamster culture, and (b) a human leucocyte culture which has been exposed to H^3 TdR labeled Chinese hamster chromosomal fractions. Note the grains on several chromosomes.

Results and discussion. On autoradiographs, cells that had been exposed to labeled chromosome suspension for 20 hours showed from approximately 10 to more than 100 disintegrations per cell in the vicinity of the metaphase chromosomes (Fig. 2a, b). Chinese hamster cells and human leucocytes behaved similarly. No gross change in chromosomal structure occurred. No label was observed in the cultures exposed for 10 hours or in the cultures that had an excess of unlabeled thymidine (Table I).

After removal of the chromosomes by centrifugation, medium that had contained no cells showed no radioactivity in the scintillation counter. DNA extracted from the cells cultured for 20 hours contained between 3 and 6% of the label that had been added to the cultures.

The observed absence of radioactivity in the cell-free medium after removal of chromosomes eliminates the possibility that soluble radioactive compounds were released from the chromosomes by enzymes in the medium.

TABLE I. Results of an Experiment Measuring Incorporation of Radioactive Label from Purified Chinese Hamster Chromosomes, into the Chromosomes of a) Chinese Hamster Cells and b) Human Leucocytes. While the experiment indicates incorporation of the label into acceptor cells, the ability of non-radioactive thymidine to inhibit this incorporation makes it likely that the original DNA was first degraded to the nucleotide level.

Time in hr	Treatment of the acceptor culture of Chinese hamster cells or human leucocytes	No other addition		5×10^{-4} mg/cc unlabelled thymidine added with the labelled chromosomes	
		Mitotic index	Fraction of mitoses labelled	Mitotic index	Fraction of mitoses labelled
t = 0	Add labelled chromosomes				
5	Add colcemide				
10	Sample: a) Chinese hamster culture	36%	0	29%	0
20	Sample: a) Chinese hamster culture	47%	100%	30%	0
	b) Human leucocyte culture	28%	85%	—	—

Extracellular digestion by the growing cells is not ruled out, but phagocytosis is rendered likely by the observations of Bensch *et al*(7) on phagocytosis of DNA-protein complex.

The failure to observe any abnormal chromosomal structures in the acceptor cells, plus the observed lack of incorporation of any H^3 TdR in the presence of an excess of unlabeled thymidine suggests that the label was not incorporated in the form of poly- or oligonucleotides. This is in agreement with the *in vivo* experiments of Robinson and Brecher (12) who offered the first proof of thymidine re-utilization *in vivo* at the nucleotide level using inhibition with unlabeled thymidine. Added thymidine may have competed in the metabolic pool with the labeled thymidine from the chromosomes at some stage in the synthesis of DNA. This differs from earlier suggestions by Hamilton(10) and others(11) that DNA is incorporated as polymer. The observed failure of the 10 hour culture to incorporate label in its chromosomes is presumably due to a time lag which is greater than the ordinary G2 period. This represents the period between addition of the labeled chromosomes and incorporation of the extracted label into the newly synthesized chromosomes. It is possible that only cells in the G1 period are able to digest the chromosomes and extract the label for incorporation into the newly synthesized DNA.

Summary. (1) A method for producing a suspension of pure mitotic chromosomes of Chinese hamster cells with insignificant contamination by interphase nuclei or their fragments is described. The yield obtained is high and the chromosome morphology is well

preserved. (2) H^3 thymidine label from such chromosomes became specifically incorporated into the chromosomes of fresh Chinese hamster cells and of human leucocyte cultures. No gross alteration in the chromosomes of the receptor cells occurred, and uptake of label was inhibited by the presence of added carrier thymidine in the medium, in an amount insufficient to inhibit DNA synthesis. Therefore, most of the incorporation appears to have involved previous degradation of the DNA at least to the level of free nucleotide.

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The Susceptibility of *Blastomyces dermatitidis* to Drying. (32065)

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One of the major mycologic mysteries has been the determination of the natural reservoir of *Blastomyces dermatitidis*. Although it is assumed that this reservoir is the soil,

the only workers who have isolated this fun-

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