Experiment No.	Right nerve cut	No. of rats	Choline acetyltransferase activity, % Right hemidiaphragm	Left hemidiaphragm	Right-left
After 1 day					
1	Proximal	2	11.8	21.9	53.9
	Distal	2	16.1	29.4	54.8
2	Proximal	2	10.9	18.5	58.9
	Distal	2	9.0	15.0	60.0
3	Proximal	2	6.4	11.1	57.7
	Distal	2	7.6	11.7	65.0
Mean:	Proximal 56.8% Distal 59.9%				
After 2 days					
4	Proximal	2	5.6	24.7	22.7
	Distal	2	4.4	28.1	15.7
5	Proximal	1	5.3	22.2	23.9
	Distal	2	4.6	24.9	18.5
6	Proximal	1	4.0	16.6	24.1
	Distal	2	3.4	21.0	16.2
7	Proximal	2	4.2	21.4	19.6
	Distal	2	2.1	20.0	10.5
8	Proximal	1	11.3	35.3	32.0
	Distal	1	3.9	24.9	15.7
Mean:	Proximal 24.5% Distal 15.3%[a]				
After 3 days					
9	Proximal	2	1.4	17.4	8.0
	Distal	2	1.3	18.6	7.0
10	Proximal	2	1.0	11.4	8.8
	Distal	2	1.0	11.4	8.8
11	Proximal	2	2.9	24.4	11.4
	Distal	2	2.4	22.0	10.9
Mean:	Proximal 9.6% Distal 8.9%				

[a] $P < 0.01$.

between 23 and 34 mm. No decrease in weight of the denervated hemidiaphragm, when compared with the contralateral one, was observed during the 3 days after the denervation.

The Table summarizes the results. It can be seen that the choline acetyltransferase activity quickly decreased after denervation. Already after 3 days it was less than 10% of that of the normally innervated side. 40 days after section of the phrenic nerve HEBB et al.[5] found it to be 3.2–3.7%. The Table shows that 2 days after denervation there was a significant difference ($p < 0.01$) between hemidiaphragms denervated by proximal, and those denervated by distal section of the nerve. It is obvious that, at this stage, the acetylcholine synthesizing power of the degenerating parts of the phrenic nerve within the diaphragm decreases more rapidly when the nerve stump in connection with the muscle is short than when it is long; whether this means that material in some way required for the synthesis is transported along the axon, even when the nerve has been divided, remains a matter of speculation. It is of interest to note that on the fourth day after denervation the supersensitivity of the muscle cells towards acetylcholine is much more advanced when the peripheral nerve stump is short than when it is long[2,7].

Zusammenfassung. An narkotisierten Ratten wurde der rechte Nervus phrenicus im Thorax entweder so hoch wie möglich, oder unmittelbar oberhalb des Diaphragms durchschnitten. Zwei Tage später erwies sich die Cholinacetyltransferase-Aktivität in der rechten Hälfte dann wesentlich geringer, wenn der Nerv distal statt proximal durchschnitten wurde.

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Translocation of Donor DNA into Nucleolar Associated Chromatin of Recipient Cell in vitro

Previous studies in vivo have shown that labelled deoxyribonucleic acid (DNA) released from transfused donor lymphocytes may be incorporated into the nuclei of the recipient cells[1–3]. The evidence was derived mainly from the appearance of label in differentiated cells that did not synthesize DNA and further from the uneven distribution of label in the nucleus as well as in the cytoplasm of the recipient cells. The data suggested that some amount of macromolecular DNA of the donor was incorporated into the recipient cells without previous degradation to acid-soluble precursors. On the other hand, the re-utilization of DNA degradation products was definitely demonstrated in vivo by ROBINSON and BRECHER[4] and by BRYANT[5]. Probably both DNA and its metabolites may be re-utilized simultaneously, depending upon the type of experiment (see LEDOUX[6] for review). The exact identification of each type of re-utilization seems to be very difficult under in vivo conditions, especially if the re-utilization is followed in the proliferating cell population. For this reason, a system in vitro has been worked out which we shall describe in this communication.

L cells were cultivated in Roux flasks in a modified Parker 199 synthetic medium supplemented with 10% calf serum. The cells grown in monolayer were pulse-labelled for 1 h in a fresh medium containing 10 μC ^{3}H-

[1] M. HILL, Expl Cell Res. *24*, 405 (1961).
[2] M. HILL, Expl Cell Res. *28*, 21 (1962).
[3] W. O. RIEKE, J. Cell. Biol. *13*, 205 (1962).
[4] S. H. ROBINSON and G. BRECHER, Science *142*, 392 (1963).
[5] B. J. BRYANT, Expl Cell Res. *37*, 490 (1965).
[6] L. LEDOUX, in *Progress in Nucleic Acid Research and Molecular Biology* (Ed. J. N. DAVIDSON and W. E. COHN; Academic Press, New York and London 1965), vol. 4, p. 231.

thymidine/ml (14.8 C/mM; Amersham). The monolayer was detached by versene, and the cells in suspension were washed with an inactive medium and inoculated into Petri dishes with cover slips. Then 25 μg of carrier thymidine/ml medium were added. After 24 and 48 h incubation, the monolayers were fixed and covered with a stripping film Kodak AR.10. The resulting autoradiograms were stained with methyl-green pyronine.

In the autoradiograms, 3 types of cells were differentiated, i.e. cells with heavily labelled nuclei, cells with unlabelled nuclei, and cells in which the label occurred in nucleolar associated chromatin. All the label disappeared after DNase digestion. Heavy labelling of the nuclei was due to ^{3}H-thymidine incorporation during the pulse-labelling period, as was shown in the monolayers which were not chase incubated. On the other hand, the label in nucleolar associated chromatin appeared in the course of 24 and 48 h chase incubation, and was found in 6.4 and 4.0% respectively of cells which were unlabelled at the beginning of the chase period. This label was mostly distributed around 1 or 2 nucleoli, irrespective of the total number of nucleoli in the nucleus (Figure 1). Two facts argue against the view that the labelling of nucleolar associated chromatin is due to the incorporation of ^{3}H-precursors into newly synthesized DNA: (1) In control experiments it was proved that the amount of carrier thymidine in the chasing medium was sufficiently high so that the newly synthesized DNA did not yield an autoradiographic image. If carrier thymidine was omitted in the chasing medium, all the already unlabelled cells were consistently labelled at the end of the chase period. (2) With regard to our present knowledge of DNA synthesis in mammalian cells, it is difficult to admit that a pronounced DNA synthesis takes place in the nucleolar associated chromatin of some nucleoli of the nucleus, independently of the S phase.

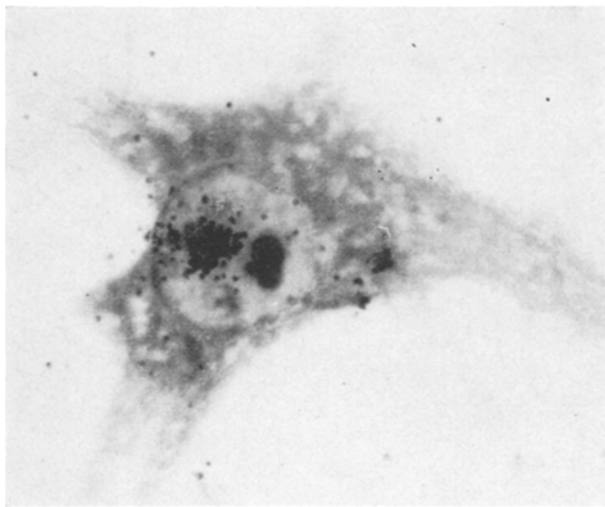

Fig. 1. L cell containing 2 distinct nucleoli. One of them reveals labelling of nucleolar associated chromatin. × 1500.

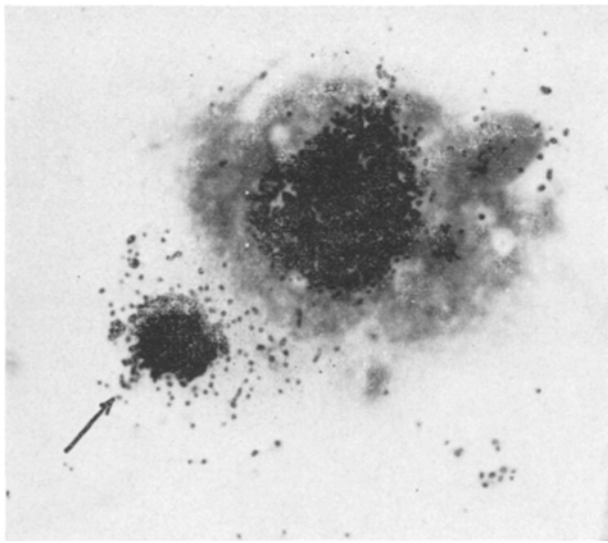

Fig. 2. Two heavily labelled L cells. One of them is intact, the other pycnotic (arrow). A corona of extracellular label is apparent around the pycnotic cell. × 1500.

Thus, the only likely source of label incorporated in nucleolar associated chromatin is DNA of heavily labelled cells. The pulse-labelled cell system may, therefore, be considered to consist of heavily labelled donor cells and unlabelled recipient cells.

It is worth noting that about 20% of cells degenerate after inoculation into Petri dishes. Heavily labelled pycnotic donor cells revealed a corona of extracellular DNA (Figure 2) similar to that described in lymphocytes[7]. In some donor cells micronuclei and chromatin bridges were found joining 2 daughter cells. Furthermore, small groups of silver grains were distributed at random over the cytoplasm of the recipient cells. Grains were often found over the cytoplasmic processes. They were absent in preparations digested with DNase. From these observations, the conclusion may be drawn that chromosome fragments and/or single DNA molecules are released from damaged cells. Thereafter, they are taken up by the neighbouring cells and partly deposited in the cytoplasm, partly accumulated in the nucleolar associated chromatin.

It seems reasonable to suggest the existence of a mechanism which concentrates the incorporated DNA in the region adjacent to the nucleolus. This hypothetical mechanism may also operate in other cell types, since Chorazy et al.[8] found the labelling over the nuclei of HeLa cells exposed to labelled chromosomes from mouse lymphatic leukemia. Evidently, more experiments are needed in order to extend these preliminary data.

Zusammenfassung. L-Zellen-Population wurde durch ^{3}H-Thymidin-Puls markiert, gewaschen und in Petri-Schalen kultiviert, nach 24- und 48-stündiger Inkubation fixiert und autoradiographisch verarbeitet. In den nach der Inkubation mit ^{3}H-Thymidin unmarkiert gebliebenen Zellen erschien um die Nukleolen eine markierte DNS. Eine Vorstellung über den DNS-Einbau wird entwickelt.

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[7] M. Hill, Expl Cell Res. *41*, 253 (1966).
[8] M. Chorazy, A. Bendich, E. Borenfreund, O. L. Ittensohn, and D. J. Hutchison, J. Cell Biol. *19*, 71 (1963).