Loss of Radioactivity from Labeled DNA of Primary Human Amnion Cells

Abstract. *Primary human amnion cultures labeled with glycine-2-C^{14} or thymidine-2-C^{14} released more radioactivity into the media than could be accounted for by cell replacement. This is interpreted as degradation of acid-insoluble polydeoxynucleotides in a living human cell.*

In a recent publication (*1*) on metabolic alterations associated with the senescence of primary human amnion cells, we have reported that 14-, 42-, or 70-day cultures each incorporated approximately similar amounts of glycine-2-C^{14} into their DNA. With uridine-2-C^{14} as tracer, the radioactivity in the RNA fractions was similar for 14-, 42-, and 70-day cultures while that in the DNA fractions was lower in the older cultures. These two findings, the fairly constant rate of incorporation of glycine into DNA of a predominantly nondividing cell population in contrast to the age-dependent decrease in the conversion and incorporation of uridine into DNA, suggest to us the simultaneous synthesis and degradation of acid-insoluble polydeoxynucleotides in these cells. To establish more firmly the existence of DNA degradation in these living cells, the following experiment was performed.

Duplicate primary human amnion cultures were labeled with glycine-2-C^{14} or thymidine-2-C^{14} for 72 hours, 14 days after explantation. After excess C^{14} substrates were removed by washing, two cultures were fractionated by the method of Schmidt-Tannhauser-Schneider into acid-soluble, lipid, RNA, DNA, and protein fractions (*2*). The results were considered as that of "zero" day. The remaining cultures were placed on a roller at 36°C. Nutrients were renewed every 3rd or 4th day. At specified intervals, two cultures selected at random were fractionated. Cell number, mitotic indices, and mitotic time were also determined at regular intervals. The rate of cell turnover was then estimated according to Leblond's formula (*3*). Technical details, including the specific activities of the C^{14} substrates, have already been described (*1*).

Table 1 shows the results of three such experiments. In each, the loss of radioactivity from DNA was considerably higher than can be accounted for by the estimated cell death. The failure to demonstrate cell replacement in experiments 2 and 3 did not imply its complete absence. It did indicate, however, low rates of cell replacement. By the autoradiographic technic, 8 and 11 percent of the cells in "zero" day cultures of experiments 1 and 2 were labeled after 1 hour exposure to thymidine-H^3 (10 μc/ml). Another "zero" day culture was treated with thymidine-2-C^{14} (0.5 μc/ml) for 3 days; 29 percent of the cell population was labeled. Labeled cells were evenly distributed throughout the cultures. Unlike cultures labeled with thymidine-H^3, those labeled with thymidine-2-C^{14} or glycine-2-C^{14} did not show visible evidence of more extensive degeneration than unlabeled controls. These autoradiographic results indicated that a certain proportion of randomly distributed cells in the primary amnion culture were incorporating the radionuclide from thymidine; this is similar to other asynchronous cell populations except for the lower proportion of labeled cells (*4*). Previous study on cell population dynamics (*1*) has already demonstrated the asynchronous nature of these amnion cultures with respect to the number of cells entering into mitoses.

We believe that this disproportionate loss of radioactivity from labeled DNA and loss of cells in an asynchronous cell population, and the disproportionate age-dependent changes in the incorporation of glycine-2-C^{14} and uridine-2-C^{14} (*1*) can best be explained by a hypothesis that DNA or some related acid-insoluble polydeoxyribonucleotides are being degraded in these living primary human amnion cells. This report is of some interest because DNA of a living cell is believed to be metabolically stable (*5*). Thomson, Paul, and Davidson (*6*) described a small loss in radioactivity of the DNA fractions of labeled mouse fibroblasts (strain L) that were continuously cultivated in the presence of thymidine. Without thymidine in the nutrient media, such loss was not noted. While these authors concluded cautiously "that the possibility of DNA turnover cannot be excluded," their data have been taken by others to indicate a 10 percent turnover of DNA per cell generation (*5*). Based on published results (*6*), it appears that cell turnover or the exchange reaction or both (*7*) is just as likely an explanation as DNA turnover for their observations (*8*).

Table 1. Loss of radioactive label from DNA of primary human amnion culture. All values are averages of results of duplicate cultures. Radioactivity is in counts per minute (cpm).

Day	Cell index*	MI† (%)	CR‡ (%)	Radioactivity (cpm per culture)					
				Glycine-2-C^{14}			Thymidine-2-C^{14}		
				Total	DNA‖	Recovered§	Total	DNA¶	Recovered§
				Experiment 1					
0	392			5882	395		7045	6979	
7		0.041	21	1485	185	4128	2694	2673	2395
14	401	.009	26	816	112	4981	2508	2504	3529
28	356	.015	40	360	81	5322	964	954	4169
				Experiment 2					
0	433			3592	197				
28	561	.012	0	404	95	3252			
				Experiment 3					
0	195						2896	2710	
7	277	.04	0				2917	2910	861
14	444	.03	0				2432	2424	1463
28	498	.01	0				1275	1247	2692

* Expressed as number of cells in thousands per culture. † Averages of miotic indices observed daily between the specific and preceding dates. ‡ Values for cells replaced (*CR*) are cumulative. *MI*, mitotic index; *MT*, mitotic time; t = total interval in minutes; *obs.*, observed number at the beginning of each interval; E = estimated number of cells.

$$CR = (E - obs.)/(obs. \times 100)$$
$$E = [(MI \cdot t)/MT] \text{ (av. cell no.)} + obs.$$

For example, the estimated number of cells on the 28th day of experiment 2:

$$E = 0.012\% \cdot [(60 \cdot 24 \cdot 28)/20] \cdot (497 \cdot 10^3) + (433 \cdot 10^3) = 553 \cdot 10^3$$

§ Radioactivity recovered in media; values are cumulative. || Separation of the prime moiety of DNA from the protein fraction was quite complete. Less than 1 percent of the total activity of the incorporated adenine-8-C^{14} remained in the protein fraction and not more than 0.5 percent of the total protein was found in the DNA fraction. ¶ Owing to poor separation of the pyrimidine moiety of DNA from protein, all acid insoluble radioactivity of thymidine-2-C^{14}-labeled cells was considered as DNA.

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References and Notes

1. R. S. Chang, *Arch. Internal Med.* **110**, 563 (1962).
2. D. Glick, *Methods Biochem. Anal.* **1**, 275 (1957).
3. C. P. Leblond and B. E. Walker, *Physiol. Rev.* **36**, 255 (1956).
4. L. E. Feinendegen, V. P. Bond, R. B. Painter, *Exptl. Cell Res.* **22**, 381 (1961).
5. A. E. Mirsky and S. Osawa, *Cell (Biochem. Physiol. Morphol.)*, **2**, 746 (1961).
6. R. Y. Thomson, J. Paul, J. N. Davidson, *Biochem. J.* **69**, 553 (1958).
7. S. Zamenhof, B. Reiner, R. DeGiovanni, K. Rich, *J. Biol. Chem.* **219**, 165 (1956); J. S. Krakow, C. Contsageorgopoulous, E. S. Canellakis, *Biochem. Biophys. Res. Commun.* **5**, 477 (1961).

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Plasma Corticosteroids: Changes in Concentration after Stimulation of Hippocampus and Amygdala

Abstract. *Plasma 17-OH corticosteroid levels were determined before stimulation and at various intervals after stimulation in these limbic sites in patients in whom implanted electrodes had been placed during evaluation for surgical treatment of psychomotor epilepsy. Stimulation of the amygdala was followed by an elevation of plasma 17-hydroxycorticosteroids. Hippocampal stimulation resulted in a decreased corticosteroid level, followed in two instances by a secondary elevation.*

Selected patients under consideration for surgical treatment of uncontrollable, severe psychomotor seizures are being studied with depth electrodes at this medical center (*1*). Rigidly supported bipolar electrodes, electrically insulated except at the tips, are stereotaxically implanted in deep temporal lobe sites by the use of radiologically defined landmarks within the skull (*2*). They are maintained in position for 3 to 4 weeks, during which time extensive evaluation procedures, including electrical stimulation in the depth sites, are conducted. The necessity for stimulation during the clinical evaluation of these patients presented the opportunity to examine in man the possible influence of these limbic structures on the pituitary-adrenal system. Observations on laboratory animals, including primates, have indicated in general that stimulation of the amygdaloid nucleus activates the stress mechanism, whereas hippocampal stimulation inhibits it (*3*, *4*).

Observations were made on four patients, 2 to 3 weeks after electrode implantation. Blood samples (10 ml) were withdrawn from a peripheral (antecubital) vein in two patients and from a catheter threaded more centrally into the subclavian vein in two others. A 60-minute interval between catheter placement and collection of the control sample was allowed to elapse, during which the patient reclined quietly in bed. After the collection of several control samples, brain stimulation was carried out. Blood was collected at intervals after a 30-second train of unidirectional square wave pulses (5 volts, 10 per second, 1 msec duration). Samples were analyzed for plasma 17-OH corticosteroid level by the method of Peterson *et al.* (*5*) (phenylhydrazine reagent was increased from 0.2 to 0.4 ml per tube). Standard deviation for the method is ±8 percent for most of the range of values. Stimulation at these parameters in the subjects studied failed to evoke detectable subjective or behavioral alterations. Brief evoked responses but no after-discharge or persistent paroxysmal activity appeared in the electrical recordings from other depth sites.

Stimulation of the amygdala (see Table 1) was followed in every instance by an increase of 17-OH corticosteroids in the blood. This is consistent with a facilitation of the pituitary-adrenal stress mechanism. This finding is consistent with the observations in laboratory animals for stimulation of the baso-medial portion of the amygdaloid nucleus (*3*). Evaluation of the significance of these observations for a functional dichotomy between baso-lateral and baso-medial amygdala suggested by studies with animals (*3*) must await histological analysis of the electrode placement in the human subjects. The immediacy of this effect argues against the possibility of a decrease in the rate of corticoid removal by the liver being responsible for the change.

Stimulation of the hippocampus resulted in a moderate initial decrease in steroid level in all subjects. In one patient (No. 4) this decrease was followed approximately 30 minutes later by an increase above control levels, which suggests that a compensatory mechanism had been activated.

These observations with human subjects support the view that the structures of the limbic system of the brain are closely related to the pituitary-adrenal stress response mechanism (*6*). The precise anatomical implications of the study await histologic analysis of the removed temporal lobe specimens.

Table 1. Corticoid response to limbic stimulation.

Location of stimulation (x-ray approximate)	17-OH corticosteroid level (μg %)					Change (%)
	Control	Minutes after stimulus train				
		0 to 5	5 to 15	15 to 30	30 to 60	
	Patient No. 1 (blood from peripheral vein)					
Pes hippocampi	31.4				21.7	−28
	Patient No. 2 (blood from peripheral vein)					
Amygdala	20.9				23.5	+12
	Patient No. 3 (central veinous blood)					
R–Pes hippocampi	16.2	3.9	2.7	1.9	12.7	−88
L–Pes hippocampi	12.7		1.3	4.6	9.2	−90
R–Amygdala	9.2		33.6	32.4	17.4	+360
L–Amygdala	17.4		40.5		23.2	+232
	Patient No. 4 (central veinous blood)					
L–Amygdala	4.0	9.5	11.5	16.8	4.5	+415
L–Ant. hippocampus	7.3	6.0	6.0	7.1	16.1	−18 +230
L–Pes hippocampi	1.0	3.4	0.0	0.0	16.7	−100 +1600

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References and Notes

1. R. D. Walter and P. H. Crandall, *Electroencephalog. Clin. Neurophysiol.*, in press.
2. Neurosurgical procedures were devised and performed by R. W. Rand and P. H. Crandall.
3. M. A. Slusher and J. E. Hyde, *Endocrinology* **69**, 1080 (1961).
4. J. W. Mason, *Am. J. Physiol.* **196**, 44 (1959); E. Endroczi and K. Lissak, *Acta Physiol. Acad. Sci. Hung.* **17**, 39 (1960).
5. R. E. Peterson, A. Karrer, S. L. Guerra, *Anal. Chem.* **29**, 144 (1957).

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